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REV. ROUM. BIOL. – ZOOLOGIE, TOME 18, No 6, p. 391–466, BUCAREST, 1973

NEW AFRICAN SPECIES OF *EUCLASTA* (LEPIDOPTERA, PYRAUSTINAE)

BY

AURELIAN POPESCU-GORJ and ALIN CONSTANTINESCU

Two species proceeding from Africa, belonging to the genus *Euclasta* Led. are described. Detailed description of genital armature in both ♂♂ and ♀♀ are given and some zoogeographical comments are also presented.

The abundant material belonging to the genus *Euclasta* Led. (Lepidoptera, Pyraustinae) we received from the British Museum and from the Pretoria Transvaal Museum (Republic of South Africa) allowed us to identify some new species. One of these is largely spread on the African continent, frequently taken for *Euclasta splendidalis* Herrich Schäffer, and another one seems to be located only in the South Africa, also frequently taken for *Euclasta warreni* Distant. We have been able to make this identification only after a previous study of the types belonging to the main species of the *Euclasta* genus and only after having obtained a broad picture on the world distribution of the species belonging to this interesting genus.

In the following, we give a detailed description of these new species which we dedicated, the former to pay our respects to Dr. Lájos Vári, prominent lepidopterologist at the Transvaal Museum of Pretoria, and the latter, to Dr. Paul E.S. Whalley, distinguished researcher at the Entomology Department of the British Museum; both of them gave unlimited assistance to our researches.

EUCLASTA VARII nova sp.

External characters: frons bearing dense, light brown scales, evenly arranged. On the vertex they are fine and long, bi- or tridenticulate and erect, protecting 2 black ocelli, situated behind antennae.

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Median portion of head and frons, towards inner margins of eyes, are marked by a shiny white-creamy line. Filiform and long antennae exceed apex of forewings. Sensory face ciliated (in ♂♂ cilia somewhat longer than in ♀♀), delimited by a white-creamy stripe, while the dorsal face is brown and each segment covered by scales; basal segment pear-shaped. Labial palpi very short and porect, broad, with distal end slightly curved downwards and acute; colour similar to the other species of the genus. Maxillary palpi short and narrow. Eyes diameter enters approximately 1.72 times into the length of labial palpi; the width of these enters approximately 2.16 times into their length. Patagial collar shiny white-creamy on the dorsal part and slightly brown on the lateral parts.

Colour of tegulae, thorax and abdomen, just like in the other species of the genus.

Forewings light brown, elongate and somewhat narrower than in *E. defamatalis* Walker which it resembles in appearance. In apical region, *costa* slightly bent. Pattern and colour of forewings, similar to those in *E. defamatalis*, the inferior half of disc-cell being covered with shining white scales, whilst the other half is provided with brown scales, separated from the shiny white portion by an almost uninterrupted brown-black line. Hindwings hyaline, pale brown with a somewhat broader infuscate area in the apical portion.

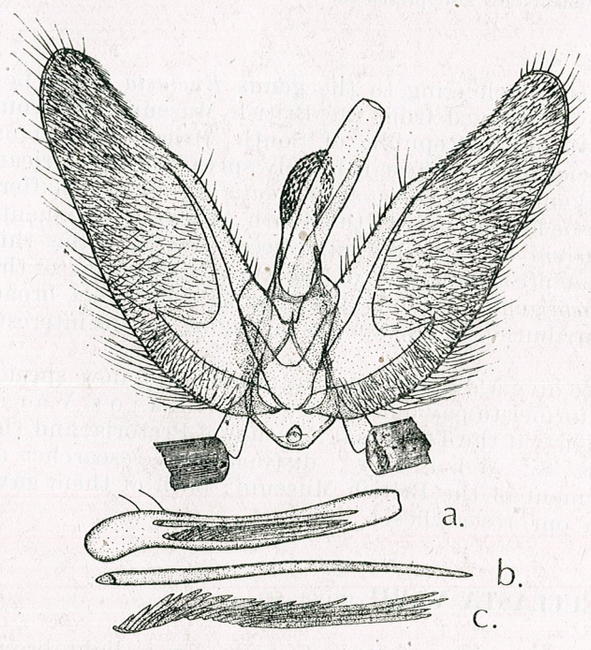

Fig. 1. — *Euclasta varii* sp. n. — ♂ genital armature; *a*, aedeagus with the 2 characteristic cornuti; *b*, even, acicular cornutus; *c*, spiny cornutus — slide no. 307 (*Holotype*), MALAWI: Mt. Mlanje (Nyasaland) 17.IV.1913 (S.A. Neave) — in the *British Museum, Natural History.*

Euclasta varii nova sp. measures: ♂♂ specimens, expanse of 23—28mm. (mostly 25—27 mm.) and the ♂♂ specimens, 24—31 mm. (mostly 27—29 mm.); in a runt specimen the expanse is only 20 mm. The ♀♀ specimens of Lybia origin, also mostly measure 24—25 mm.

Male genitalia (Fig. 1, holotype — slide no. 307). *Tegumen* convex and tapering, presents a large notch on anterior portion. *Uncus* slightly elongate with distal end club-shaped. Dorsal part heavily convex, covered with diverging scales, like spiny lamellae. *Tuba analis* slightly chitinized, considerably overlaps distal end of uncus. *Gnathos* well chitinized, U-shaped, consisting of two narrow arms, anteriorly slightly broadened and joined by a transversal, slightly curved bar. *Valvae* membranous, antero-posteriorly elongate, broadened in the median portion; breadth enters 3—10 times approx. into their length. *Costa*, beyond the half of valve, tilted towards distal, rounded end, valves tapering somewhat more than in *E. defamatalis*. Inner face of valve lined with a membrane on which numerous fine, long hairs are to be found, directed towards the base of valves. This membrane is interrupted towards the basis so as to expose a Y-shaped space with unequal arms. *Sacculus* well chitinized, short, triangular. *Vinculum* with a sclerite presenting at the end, a plate on which *coremata* is inserted; this latter consists of a tuft of fine, very long hairs, overlapping the length of valves. *Aedeagus* (Fig. 1 *a*) elongate, cylindrical, slightly inflated in its proximal portion (*coecum-penis*); *ductus ejaculatorius* bears 2 characteristic *cornuti* (Fig. 1 *b* and *c*) : one even, acicular, slightly curved towards distal end (*b*) and another one, spiny, somewhat shorter than the acicular one, slightly contorted towards proximal end, in contrast with distal end where spines are more elongate. Position and shape of these cornuti are specific to each species. Also in this species, the spiny cornutus is frequently absent; sometimes found in doublet, in ovigerous females (either one in the *ductus bursae* and a second one in *bursa copulatrix*, or both having reached the *bursa copulatrix*), as females present an obvious polyandry. *Fultura inferior* consists of 2 diverging lobes.

Female genitalia (Fig. 2, allotype — slide no. 306). *Bursa copulatrix* slightly egg-shaped, presents concentric folds and a spectacles-shaped *signum*, specific to the genus. Ductus bursae, after one coil, the portion where opens *bula seminalis*, begins to inflate to a frustum of a cone; up to its half it gets the normal size. Over more than a half of the inflated portion, ductus presents a chitinization, like an elongate funnel, opening towards proximal portion, which is characteristic of this species. After narrowing, ductus remains membranous up to the distal end where it slightly inflates, its walls presenting a few longitudinal folds. Then, it suddenly narrows and opens into the *antrum*; in the narrow portion there is a chitinized plate, slightly oval, with close and rounded margins. *Antrum*, in its proximal part, on the outer face, bears fine and rare spinules, and near the *ostium bursae*, a ring of very dense, fine spinules. Anal papillae and apophyses similar to the other species of the genus.

Material examined : 39 spec. ♂♂, 37 spec. ♀♀ and 1 spec. without abdomen, proceeding from different African countries, namely :

Holotype ♂ : MALAWI : Mt. Mlanje (Nyasaland) 17.IV. 1913 (leg. S.A. Neave) — slide no. 307 — in the *British Museum, Natural History.*

Allotype ♀ : MALAWI: Mt. Mlanje, 2300 ft. (Nyasaland) 11.VIII.1913 (leg. S.A. Neave) — slide no. 306 — in the *British Museum, Natural History.*

Paratypes 38 spec. ♂♂:

SOUTH AFRICA : 1 spec. Belvedere, C.P.V. 1921 (Dr. H.G.Breijer); 1 spec. Durban (ex coll. Clark, A.J.T. Janse); 1 spec. Potgietersrust Powel (Transvaal) I. 1939 (coll. Janse) — in the *Transvaal Mus. Pretoria*; 6 spec. Pretoria N :20.I.1917 (C.J. Swierstra) — in the *Nat. Mus. South Rhodesia*; 18.II.1939; 15.V. 1937; 30.IX.1937 — slide no. 383; 25.X. 1937 (2 spec.) (all G. van Soon); 1 spec. Pretoria 1. IV.1969 (L. Vári); 3 spec. Skukuza (Kruger Nat. Park Survey) 26—28.IV.1968 (Potgieter et Goode) (2 spec.)— slide no. 384 and 7.V.1970 (Vári et Potgieter); 1 spec. Punda Milia (in northern Kruger Nat. Park Survey) 4—5.V.1970 (Vári et Potgieter) — all in the *Transvaal Mus. Pretoria*; MOZAMBIQUE : 1 spec. Laurenço-Marque V.1907 (Dr.G. Audéoud) — slide no. 395—in the *Mus. de Genève*; RHODESIA: 1 spec. Vumba 2.V. 1968 (B.D. Barnes); 1 spec. Salisbury Expt. Stn IV. 1956; 1 spec. Victoria Falls IX. 1957-in the *Nat. Mus. S. Rhodesia*; 1 spec. Nankie XII. 1964 — in the *Mus. Malawi*; ANGOLA : 1 spec. Mt. Moco, Luimbale 1800—1900 m. 15.III.1934 (Dr. K. Jordan) — slide no. 320; ZAIRE : 1. spec. Kassai district (Taymans) — in the *British Mus. Nat. History*; 2 spec. Tsinkolobwe (Ht. Katanga) 2.I.1931 and 5.VI.1931 (J. Romieux) — slide no. 379 — in the *Mus. de Genève*; 1 spec. Elisabethville VI.1938 (Dr. Seydal) — in the *Transvaal Mus. Pretoria*; 1 spec. Kwamouth, VI.1921 (Lebrun); 1 spec. Eala, I. 1953 (J. Ghesquière)—in the *Mus. du Congo-Tervuren*; MALAWI : 3 spec. Mt. Mlanje (Nyasaland) 21.I.1914; 29.I.1914; 25.III.1913; 1 spec. Shire Valley, Mwanza Riv. 600 ft.4.VIII. 1913 — slide no. 301; 1 spec. S.W. Lake Chilva 12.I.1914 — slide no.303; 1 spec. Ruo Valley, 1000 ft. 13.VII.1913 (all S.A. Neave) — in the *British Mus. Nat. History*; 1 spec. Nkata bay (Nyasaland) XII.1961— in the *Nat. Mus. S. Rhodesia*; ZAMBIA : 1 spec. bks. Loangwa. Lat. 14.50 S., about 1700 ft. 29.VI.1905 (S.A. Neave) — in the *British Mus. Nat. History*; 1 spec. Katambora IV.1962 — in the *Nat. Mus. S. Rhodesia*; TANZANIA : 1 spec. Dar-es-Salaam — slide no. 302 — in the *British Mus. Nat. History*; SENEGAL : 1 spec. Kaolack (coll. le Moult) — slide no. 305 — in the *Mus. de Paris*; YEMEN : 1 spec. F. Muir Reg. (Aden), 3.XI.1900 — slide no. 300 — in the *British Mus. Nat. History*.

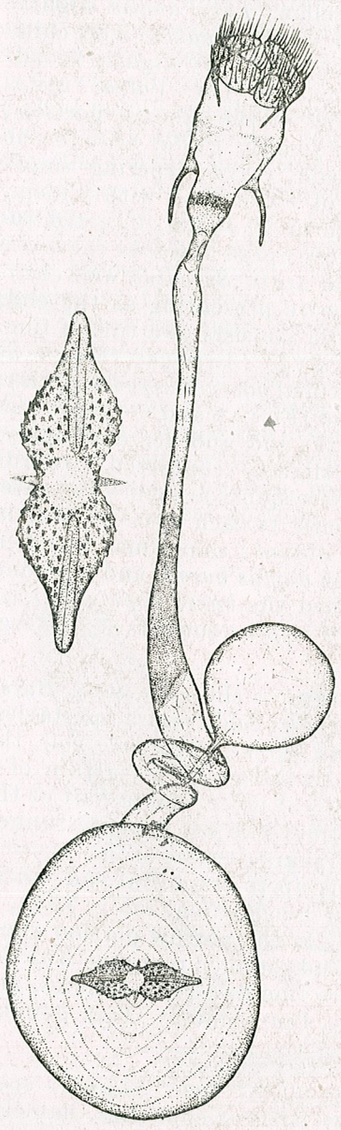

Fig. 2. — *Euclasta varii* sp. n. — ♀ genital armature; slide no. 306 (Allotype), MALAWI: Mt. Mlanje, 2300 ft. (Nyasaland) 11.VIII.1913 (S.A. Neave)—in the *British Museum, Natural History.*

Paratypes 38 spec. ♀♀:

SOUTH AFRICA : 1 spec. Pinetown (Natal) I. 1909 — slide no. 290 — in the *British Mus. Nat. History*; 1 spec. Pretoria N. 18 II.1939 (G.v. Son); 4 spec. Pretoria 13.I.1924; 22.II.1937; 9.VI.1924 (G.v.Son) (A.T. Janse) — slide nos. 393 and 394; 13.IX.1937 (G.v.Son); 1 spec. Crocodile Bridge (S. Kruger N. Park) 25.III.1952 (Jansen et Vári); 1 spec. Skukuza (K.N.P. Survey) 7.V.1970 (Vári et Potgieter) — all in the *Transvaal Mus. Pretoria*; ZAIRE : 1 spec. Elisabethville 25.III.1934 (Dr. Bourguignon) — slide no. 304 — in the *Mus. du Congo-Tervuren*; MALAWI : 1 spec. Upp. Luangwa R., Btwn. Luwumbu et Mwailesi Riv's 11.VIII. 1910 (S.A. Neave) — slide no. 380; 1 spec. Mt. Mlanje (Nyasaland) 22.III. 1913 (S.A. Neave); 1 spec. Shire Valley (Nyasaland), Mwanza Riv., 600 ft. 5.VIII. 1913 (S.A. Neave); TANZANIA : 1 spec. E. Africa, 1893 — all in the *British Mus. Nat. History*; RHODESIA : 2 spec. Gatooma II-III. 1968 (leg. C. Cottrell) — in the *Nat. Mus. S. Rhodesia* and VI.1956 — in the *Mus. Malawi*; 3 spec. Lindi, Ndanda (Tanganyika), 300 m. 7. VIII.1952 (leg. Lindemann und Pavlitzki); 3.XII.1958; 5.XII.1958 (leg. Ch. Lindemann) — slide no. 378 — in the *Staatsslg. München*; SIERRA LEONE : 1 spec. W. Africa (Sierra Leone) XII.1967 (R.J. Revell) — slide no. 319; ALGERIA : 1 spec. Hammam-es-Salahin 24.IV.1904 (Walsingham) — slide no. 335; 1 spec. El-Outaya VI.1910 (V. Faroult) (coll. Paravicini) — slide no. 308, all in the *British Mus. Nat. History*; TUNISIA : 2 spec. Gafsa 26.IV; 28.IV (coll. Caradja) — slide no. 2501 — in the *Mus. Gr. Antipa*; 3 spec. Nefta 6.V.1929 (2 spec.); 13.IX.1932 (coll. C. Dumont) — slide no. 382 — in the *Mus. de Paris*; LIBYA : 11 spec. Sidi-Mesri (Tripolitania) : 21—31.III.1924 (Romei) (4 spec. coll. Rothschild Bequest and 1 spec. coll. Paravicini) — slide no. 318; 2.VII.1924 (2 spec. one of which belongs to the *Mus. Karlsruhe* — slide no. 3022); 3.VII.1924; 4.VII.1924; 6.VII.1924 — slide no. 309; 3.VIII.1924 (Ederli) (coll. Rothschild Bequest); YEMEN : 1 spec. Jebel Jihaf (W. Aden), cca. 7100 ft., X. 1937 (H. Scott et E.B. Britton) — slide no. 291 — all in the *British Mus. Nat. History*.

1 spec. without abdomen : MOZAMBIQUE : 1 spec. 12.VII.1893 (Dr. Ansorge) — in the *British Mus. Nat. History*.

Biology : unknown.

Distribution : *Euclasta varii* nova sp. is largely spread on the African continent, the material examined by us proceeding from : Republic of South Africa, Mozambique, Rhodesia, Angola, Zaire, Malawi, Zambia, Tanzania, Senegal, Sierra Leone, Algeria, Tunisia and Libya; also occurring in Yemen, in the Arabian Peninsula. Absent in Madagascar as well as in the isles on the Atlantic coast of Africa.

EUCLASTA PAULI nova sp.

External characters : frons bearing dense, dark brown-grey scales, delimited all around by a wide shining-white stripe, median stripe on the frons absent or very slightly marked. Filiform antennae just exceeding the expanse of forewings. Labial palpi long, with basal portion and ventral margin largely covered with shining-white scales; median portion also covered with shining-white scales alternating with brown-grey ones; the remaining parts, covered only with brown-grey scales. Distal end of labial palpi acute; eyes diameter enters 2.40 times approx. into palp length, and palp width enters 2.80 times approx. into their length. Maxillary palpi elongate, somewhat shorter than in *E.warreni* Dist. Proboscis also provided on its outer surface with shining-white scales.

Tegullae brown-grey with shiny-white margins.

Forewings brown-grey, elongate, with costa slightly curved towards apex; disc-cell almost totally covered with shiny-white scales, with a round black spot on the upper part of distal end; the spot is supported by the distal end of a continuous black line, which severs the subcostal space from the dorsal one, and which extends, in separate fragments, up to the apical portion. In the submedian part of distal end of the disc-cell, there is a brown-black stripe, slightly curved, externally delimited by a fine shiny-white line; the space between this bent line and the outer margin of the wing, somewhat broader than in *E. warreni* Dist., a very resembling species.

Hindwings subhyaline, with a slight purplish-blue iridescence and a grey area, broader in the apical portion, decreasingly descending towards anal angle, its inner margin being more diffuse, not so distinct as in *E. warreni*.

Abdomen also brown-grey, shorter and larger than in *Euclasta* species belonging to the *defamatalis* group (i.e. those with brown wings). In females pattern and colour are identical.

Euclasta pauli nova sp. measures : spec. ♂♂ 28—31 mm. (mostly 31 mm.) and spec. ♀♀ 28—34 mm. (mostly 31—33 mm.).

Male genitalia (Fig. 3, *holotype* — slide no. 396). *Uncus* shorter and rounded, dorsal part heavily provided with divided scales, like spiny lamellae. *Tuba analis*, fairly membranous, not overlapping uncus. *Gnathos* well chitinized with the same U-shape. *Valvae* membranous, antero-posteriorly elongate, slightly broadened in their median portion, almost oval in shape. In the median portion *costa* is slightly tilted towards distal end, which is rounded and less narrow as compared with other species. Valve breadth enters 3.10 times approx. into its length, a little shorter than in *E. warreni* Dist. *Coremata* made up of a tuft of fine and very long hairs, projected beyond length of valvae. *Aedeagus* (Fig. 3 *a*) elongate, cylindrical and slightly inflated in the proximal part, whilst the distal part is narrower and slightly curved; *ductus ejaculatorius* bears 2 specific *cornuti* : an even one, slightly curved towards distal end, which is acute and presents at the proximal end an aperture confined (towards distal part) by a membrane which appears like a pointed hook, in profile view (Fig. 3 *b*). In contrast with other *Euclasta* species, this cornutus is longer than the second, spiny one (Fig. 3 *c*), the spines on the distal end being much longer; also in this species the spiny cornutus is lacking.

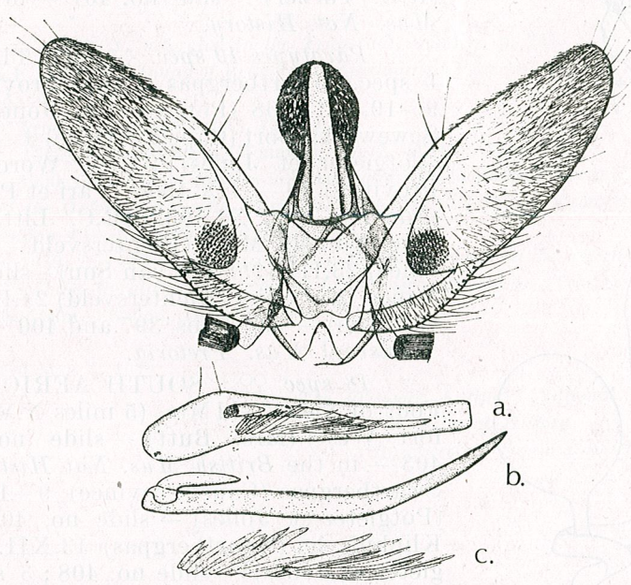

Fig. 3. — *Euclasta pauli* sp. n. — ♂ genital armature; *a*, aedeagus with the 2 characteristic cornuti; *b*, even, acicular cornutus; *c*, spiny cornutus — slide no. 396 (*Holotype*), SOUTH AFRICA : Nuisabies (Richtersveld) 24.III.1958 (G. van Son) (Cape Province distr.) — in the *Transvaal Museum Pretoria*.

Female genitalia (Fig. 4, *allotype* — slide no. 401). *Bursa copulatrix* slightly oval, with many concentric folds and a large spectacles-like *signum*, with lateral projections shorter than in other species. *Ductus bursae* membranous, after one coil, where opens *bula seminalis*, almost immediately inflates on nearly its whole median portion, the walls of which are almost entirely slightly sclerotized; after becoming again membranous it narrows a little, to inflate again, a portion where the walls bear longitudinal folds; the end towards the *antrum* bears a sclerite, like a sheet of paper with close and rounded margins. *Antrum* inflated, cup-shaped, with outer walls almost entirely provided with very fine, dense spinules. Anal papillae and apophyses similar to those of the other species belonging to the genus.

Material examined: 11 spec. ♂♂, 19 spec. ♀♀ and 3 spec. without abdomen.

Holotype ♂: SOUTH AFRICA: Nuisabies (Richtersveld) 24.III.1958 (G. van Son) (Cape Province Distr.) — slide no. 396 — in the *Transvaal Mus. Pretoria.*

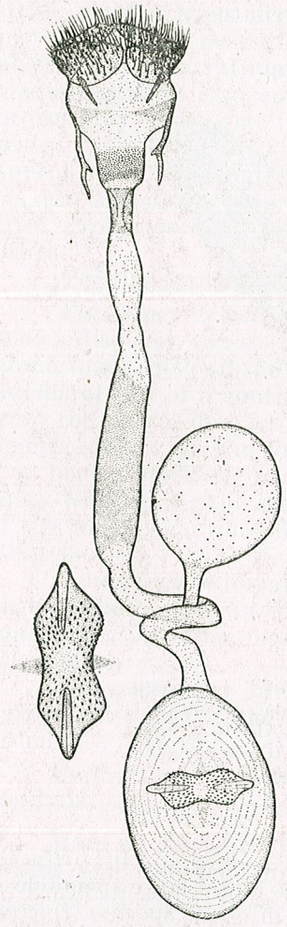

Fig. 4. — *Euclasta pauli* sp. n. — ♀ genital armature; slide no. 401 (Allotype), SOUTH AFRICA: Prince Albert Rd. (Cape Province distr.) XI.1931 (R.E. Turner) — in the *British Museum, National History.*

Allotype ♀: SOUTH AFRICA: Prince Albert Rd. (Cape Province Distr.) XI.1931 (R.E. Turner) — slide no. 401 — in the *British Mus. Nat. History.*

Paratypes 10 spec. ♂♂: SOUTH AFRICA: 1 spec. Swartbergpas (Cape Province distr.) 9—19.XII.1968 (Potgieter et Jones); 3 spec. Seweweekspoort (Cape Province) 4—6.XII.1968 (Potgieter et Jones); 2 spec. Worcester (Cape Province) 14—21.X.1966 (Vári et Potgieter) — slide no. 398; XI.1962 (R.C. Littlewood); 1 spec. Numees Mine (Richtersveld — Cape Province) 23.III.1958 (G. van Son)—slide no. 399; 3 spec. Nuisabies (Richtersveld) 24.III.1958 (G. van Son) — slide nos 397 and 400 — all in the *Transvaal Mus. Pretoria.*

18 spec ♀♀: SOUTH AFRICA: 2 spec. Foot of Nieuwveld Mts. (5 miles N.W. of Beaufort West) (Mrs. Butt) — slide nos 402 and 403 — in the *British Mus. Nat. History*; 1 spec. Swartbergpas (Cape Province) 9—19.XII.1968 (Potgieter et Jones) — slide no. 405; 1 spec. Kliphuisvlei (Swartbergpas) 13.XII.1968 (Potgieter et Jones) — slide no. 408; 3 spec. Seweweekspoort (Cape Province) 4—6.XII.1968 (Potgieter et Jones); 4 spec. Numees Mine (Richtersveld) 23.III.1958 (G. van Son) — slide no. 404; 2 spec. Nuisabies (Richtersveld) 24.III.1958 (G. van Son); 2 spec. Willowmore (Cape Province) 10.IV.1929 (G. van Son) and X. 1915 (coll. Janse); 1 spec. Swellendam (Cape Province) 9—14.XII.1931; 1 spec. Lonridge Farm (Plettemberg Bay, Cape Province) 13—17.III.1956 (A.J. Duke); 1 spec. Oudtshoorn (Cape Province) 18.VIII.1949 (C.G.C. Dickson) — all in the *Transvaal Mus. Pretoria.*

3 *spec. without abdomen*: 1 spec. Swartbergpas 9—19.XII.1968 (Potgieter et Jones); 1 spec. Seweweekspoort 4 — 6.XII.1968 (Potgieter et Jones); 1 spec. Stormsriviermond (Coast Nat. Park) 4.XI.1967 (Vári et Potgieter) — all in the *Transvaal Mus. Pretoria.*

Biology: first stages and ecology unknown.

Distribution: according to available data *Euclasta pauli* sp. n. seems to be located in South Africa, exclusively, south of the tropic of Capricorn, being captured from different localities in the Republic of South Africa (Transvaal and Cape Province districts). From the zoogeographical viewpoint, *E. pauli* is a south Ethiopian element.

REFERENCES

1. Distant W.L., 1892, *A naturalist in the Transvaal*, London, p. 241.
2. Hampson G.F., 1896, *The fauna of British India including Ceylon and Burma. Moths*, London, **4**, 363—364.
3. — , 1898, Proc. Zool. Soc. London, 751—752.
4. Lederer J., 1863, Wiener Entom. Monatschrift., **7**, 34, 132, 189.
5. Swinhoe C., 1900, *Catalogue of Eastern and Australian Lepidoptera Heterocera in the collection of the Oxford University Museum. Part VII: Noctuina, Geometrina and Pyralidina.* Clarendon Press, Oxford, 514.
6. Walker F., 1859, *List of the specimens of Lepidopterous insects in the collection of the British Museum, Pyralides (Catalogue of Lepidoptera Heterocera).* Trustees of the British Museum, London, **18**, 544—545.

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REPRODUCTION AND POSTEMBRYONIC ONTOGENETIC DEVELOPMENT IN *LIGIDIUM HYPNORUM* (CUVIER) AND *TRICHONISCUS PUSILLUS* (BRANDT 1833) (CRUSTACEA, ISOPODA)

BY

NICOLAE TOMESCU

In the paper the author describes the aspects of the postembryonic ontogenetic development and reproduction in two terrestrial isopod species : *Ligidium hypnorum* and *Trichoniscus pusillus*. The gestation periods of the species and of the females, the number of eggs layed by a female and the number of hatchings per age classes are established. With the ontogenetic development, the starting point of sexual differentiation and the stage of the animals of the two generations when entering the diapause are shown. There are also described the morphologic characters of each ontogenetic development stage in both species.

Data concerning the reproduction of these two species of isopods are few and incomplete, and their postembryonic ontogenetic development was not studied till now. The results of some similar researches [11], [13] previously published, convinced us of the necessity of making a study of the ontogenetic development in most isopod species.

MATERIAL AND METHOD

As biologic material we used animals collected in field from 1969 to 1970, the aim being the study of the dynamics of some isopod populations. For the reproduction period (May — September) the samples were taken at 15 days intervals, and afterwards at an interval of a month. To obtain larvae immediately after hatching out we brought gestant females in laboratory, keeping them until the larvae hatching. Out of *Ligidium hypnorum* species we studied 656 individuals (172 males, 205 females, and 279 larvae), and out of *Trichoniscus pusillus* spe-

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cies 924 individuals (253 males, 425 females, and 246 larvae). For the postembryonic development study our observations were made with the stereomicroscope, on each individual separately, and out of 60 and 75 males we made microscopic preparations with appendices which represent the secondary sexual characters with taxonomic value. The big number of the investigated individuals as well as the material collected during an entire year made it possible to observe the evolution of characters of each species during the ontogenesis. We made also comparative observations on the variabilities of some phenotypic characters.

RESULTS AND DISCUSSIONS

Reproduction. Ligidium hypnorum. The gestation period with this species is from May till August (about 90 — 100 days), and the gestation period of the female is of 40 — 45 days. In the month of May we found as gestant only the females belonging to the age class of 2—3 years, with the body size of 9—10 mm. The number of eggs layed by these females at their first laying is of 16 — 25, frequently 16 — 17. The larvae from the laying of the month of May are hatching out at the end of June and the beginning of July. About the middle of July, few females of 2—3 years, surviving the first laying, are laying for the second time, and the larvae are hatching out about the middle of August. The 16th of August we found females still having incubation sack, and the 20th of August the postnatal moulting had already taken place. In June, the 1 — 2-year-old females, with body size of 7 — 9 mm. were gestant. These females are laying 10 — 20 eggs, frequently 13 — 16. The 1 — 2-year-old females are also laying twice consecutively. The incubation time with the second generation is shorter. In this warm period (July — August) the thermal constant is realized in a shorter time as compared to the first generation. Females belonging to the age class of 0 — 1 year, and having a body size of 5 — 7 mm. are laying only once, in the first year of life, at the end of June. The females are laying 6 — 10 eggs. The number of females of 0 — 1 year and of 5 mm. was reduced; they have not layed in their first year of life.

Trichoniscus pusillus. With *T. pusillus* the gestation period of the species begins in May and lasts till September (about 130—140 days), and the gestation period of the female is of 30 — 35 days. As with the first species, the 2 — 3-year-old females, having a body size of 3 — 3.5 mm. are laying in May. A female lays 7 — 12 eggs, frequently 8 — 9. About the end of May, the 1 — 2-year-old females, of 2 — 2.8 mm. in size are also laying 6 — 10 eggs, frequently 6 — 7. In June, with the 2 — 3-year-old females the larvae are in a very advanced stage of development. These females have 2—3 consecutive layings, and those of 1 — 2 years, 2 layings. The 0 — 1-year-old females with a body size of 1.8 — 2 mm. are laying only once, 4 — 6 eggs, in June — July.

The authors who studied the aspects of reproduction in different isopod species [2], [9], [16], [17] referred to the periods when gestant females appear, as well as to the number of eggs and to the layings, making a correlation only with the animals' size. Our results show that the number of eggs and of layings of a female is depending on the size and age, but that the number of eggs is also depending on the range of laying. With

the second laying, the same females in both species have a smaller number of eggs as compared to the first laying.

Reckoning the rate of gestant females in June and July, months when females in all classes of age may normally be gestant, we found that with *Ligidium hypnorum* the rate of gestant females is of 84.2 %, and with *Trichoniscus pusillus* of 84.7 %. These prove that a quite large number of females take part in the reproduction, ensuring the species perpetuation and a high density of the population in the biotope. This fact was also established by our results, after the quantitative analysis of the samples.

Ontogenetic development. The importance of knowing the postembryonic ontogenetic development of isopod species was underlined by Vandel [14], [15]. He shows that in order to make the revision of some species and genera, or to establish phylogenetic relations among species, it is necessary to know their ontogenesis. Many organs of the isopod body have an allometric growth during the ontogenesis, presenting thus different shapes in the stages of development. These reasons as well as the results of other papers [11], [13] determined us to continue the study of postembryonic ontogenetic development in other soil isopod species, too. In our research we observed the evolution of the clearly visible phenotypic characters, which are modified from a stage to another, and especially of the male secondary sexual ones. They are fundamental criteria in delimiting the species. The genesis of the male sexual organs, representing the secondary sexual characters, is directly influenced by the androgenic gland. It co-ordinates the formation and growth of these organs. Besides the androgenic gland, an important part is played by the neurohormones secreted by the median zone of the protocerebrum. They have an antagonistic action toward the androgenic gland. The physiological mechanism of sexual differentiation in isopods was studied by many scientific researchers [1], [3], [5], [6], [7], [11], who have shown that during the ontogenesis the secondary sexual organs appear in different stages, and that their subsequent evolution occurs under the action of sexual hormones and neurohormones. The functional ratio between the androgenic gland and the protocerebrum changes during the ontogenesis, the inhibitory action of the neurohormones on the androgenic gland diminishing gradually. These physiological modifications are expressed also phenotypically and correspond to some progressive morphologic modifications of the secondary sexual organs, which are characteristic of each stage of development in animals. We continue with the description of the stages of postembryonic ontogenetic development in *Ligidium hypnorum* and *Trichoniscus pusillus.*

Ligidium hypnorum — larval stage. After the hatching the size of the larvae is of 1.8 — 2 mm. The body has a yellowish colour, under the stereomicroscope a fine net of brown pigments being observed. The head has no lobes (Fig. 1 *a*), a common feature with the adult individuals. The eyes are formed of a reduced number of ommatidia which are increasing with the animal growth. The antennary flagellum is formed of 7 segments (Fig. 1 *b*). The 7th segment of the pereion is incompletely developed, and the pereiopod 7 is lacking. The exopodites of the uropods are shorter than the endopodites; the ratio ex./en. is 0.86 (Fig. 1 *c*). During the ontogenesis the exopodite has a positive allometric growth as compar-

ed to the endopodite, surpassing it in length even in the immature stage. At 20 — 25 days after hatching the larvae reach 3 — 3.5 mm. in size. In this period the pigmentation of the body becomes more pronounced and visible with the naked eye. The pereion and the 7th pereiopod are completely developed.

Immature stage. Sexual differentiation is visible when the larvae size reaches 3.5 mm. We found the first immatures from generation I

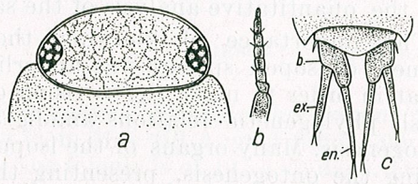

Fig. 1. — *Ligidium hypnorum — larval stage.* *a* — head; *b* — antenna; *c* — pleotelson; b. — basipodite, ex. — exopodite, en. — endopodite.

(larvae hatched in June) in the samples collected between 5 and 15 August; their size was of 3.5 — 3.8 mm. But in this period we also found recently hatched larvae from generation II. We studied their growth as well as their sexual differentiation. The immature stage is characterized by the following: the antennary flagellum is formed of 8 segments (Fig. 2 *a*), the exopodite (Fig. 2 *b*) and the endopodite (Fig. 2 *c*) of the pleopod I are resembling those of the adult as concerns the shape. They differ by the more reduced size, the exopodite is lacking both thorns, and in the endopodite there is only one. The endopodite of pleopod II is short-stem-shaped, with a round end (Fig. 2 *a*). The uropodal exopodite seems shorter than the endopodite because of the shape of the basipodite. The basipodite is shorter in the portion in which the exopodite is joined, and lengthened as a cone where the endopodite is joined (Fig. 2 *f*). The length ratio exopodite/endopodite is 1.1 to the exopodite's advantage.

Juvenile stage. The first juveniles of generation I were found by us at the beginning of September, at 50 — 60 days after hatching. The

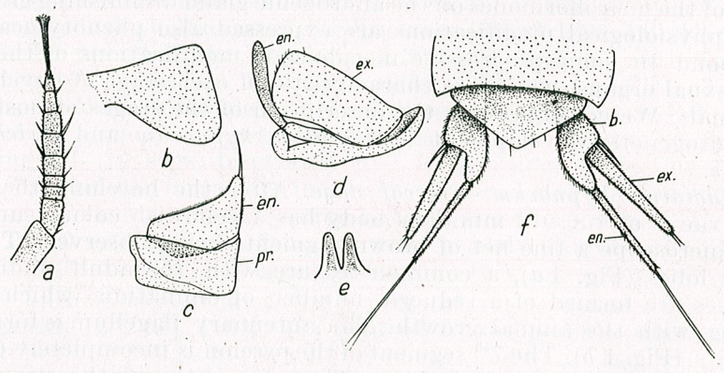

Fig. 2. — *L. hypnorum — immature stage.* *a* — antenna; *b* — ex. pl. I; *c* — en. pl. I; pr. — protopodite; *d* — pleopod II; *e* — penis; *f* — pleotelson.

juvenile features in this generation appear with the animals having 4.7 mm. in size. The body colour resembles that of the adult, that is to say finely marbled with brown and yellow spots. On the lateral side of the body there are 2 brown longitudinal strips. The antennary flagellum is formed of 9 segments (Fig. 3 *a*). In the exopodites and the endopodites of pleopod I 2 thorns appear (Fig. 3 *b*, *c*). The endopodite of pleopod II

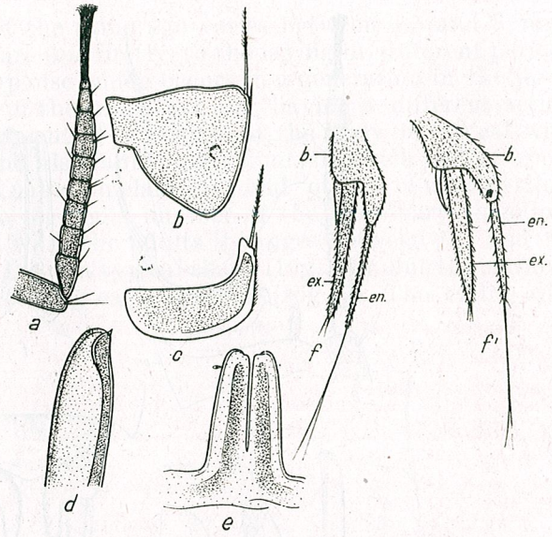

Fig. 3. — *L. hypnorum — juvenile stage.* *a* — antenna; *b* — ex. pl. I; *c* — en. pl. I; *d* — en. pl. II extremity (gonopod); *e* — penis; *f*, *f'* — uropods.

the gonopod) is as a long duct with a rounded off end, provided with a chitinous tooth in terminal position (Fig. 3 *d*). The penis is adult-like, but the ratio length/thickness is 3.8—4.0, while with the adult it is 4.5—5.5. The ratio ex./en. in uropods is 1.26 — 1.33. The extremity of the exopodite reaches the same level as that of the endopodite (Fig. 3 *f*). The individuals from generation I enter the diapause when juveniles. The males are of 5 — 5.5 mm. in size, and the females of 6 mm. The individuals hatched in August (generation II) enter the diapause as immatures and are of 3 — 4 mm. in size. As will be seen, the diapause has a different influence on the subsequent development of immature and juvenile individuals. In the month of April of the following year the male juveniles from generation I reach the size of 6 — 6.2 mm. and the antennary flagellum is formed of 10 segments. The ratio ex./en. with uropods is also of 1.3, in some individuals even smaller. With the immature males resulted from generation II, a lack of balance between growth process and sexual maturation occur in spring. Sexual maturation takes place in a faster rhythm, which is detrimental to the growth. These individuals become adult at the same time with those from generation I, but having a much smaller size. In May, the juveniles' gonopods and penis have a morphologic aspect resembling the adult (Fig. 4 *e*, *f*). The antennary flagellum is also formed of 10 segments, and the ratio ex./en. in uropods is of 1.33, too (juvenile characters).

Adult stage. Males of both generations have entire characters of adults at the beginning of June, that is to say after 10 and 11 months from hatching. Those from generation I reach the size of 6 — 7 mm. and those from generation II of 4.5 — 5.5 mm. The secondary sexual organs are utterly resembling from a morphologic point of view. But there is an obvious difference in size between the two generations. The characters of

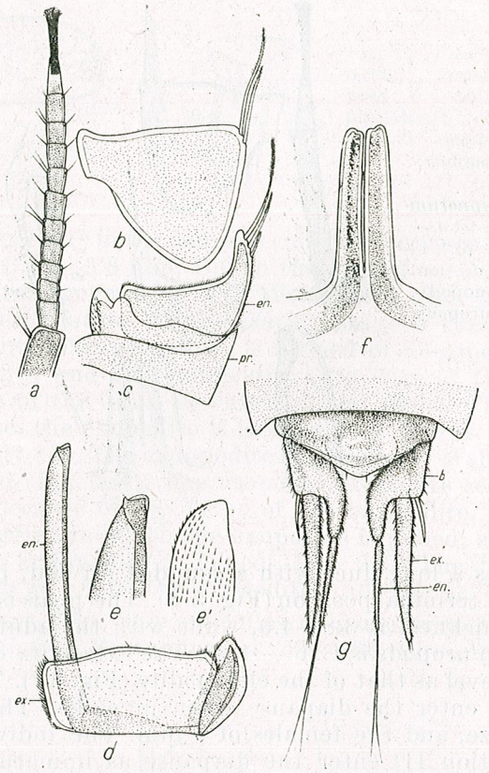

Fig. 4. — *L. hypnorum — adult stage.* *a* — antenna ; *b* — ex. pl. I ; *c* — en. pl. I ; *d* — pl. II ; *e* — enlarged extremity of en. pl. II ; *e'* — the same extremity pressed on the slide ; *f* — penis ; *g* — pleotelson.

a male adult are shown in figure 4. We notice the fact that the antennary flagellum is formed of 11 segments (Fig. 4 *a*). The en. pl. II (the gonopod) extremity has two chitinous teeth (Fig. 4 *e*). The ratio ex./en. in uropods is of 1.6. The distal end of the exopodite always exceeds, both in males and females, the distal end of the endopodite (Fig. 4 *g*). On the whole, these characters allow us an accurate distinction between adults and juveniles. When the males become adults, their growth is very slow, so that in autumn one can find in a population juveniles from generation I, which may reach and exceed in size the adults from generation II of the last

year. Knowing the juvenile and adult features, their distinction is somehow easily done and with much accuracy. This is necessary in systematic studies as well as in the ecology and the genetics of isopod populations. It is necessary to know first the stage variations, to investigate then the genotypic variations and the modifications in several populations belonging to a species with a large area of distribution. In what follows we shall mention some data on the phenotypic variations observed by us in *Ligidium hypnorum*.

The size of adults of the same age varies between 4.5 and 8 mm. The big differences in size are due firstly to the laying in different periods of the year and to the diapause which occurs in a generation in the juvenile stage and in another in the immature one, having a different action on the subsequent development. The colour of the body is typical with most animals ; but we found also adult individuals in which brown spots were predominant (accentuated melanism), and others in which yellow spots were prevalent. The segments number of the antennary flagellum has stage variations, and with the adults it ranges between 11 and 13. The exopodite of pleopod I is of a typical shape (Fig. 4 *b*), but the number and length of the thorns present great variations (Fig. 5). The endopodite

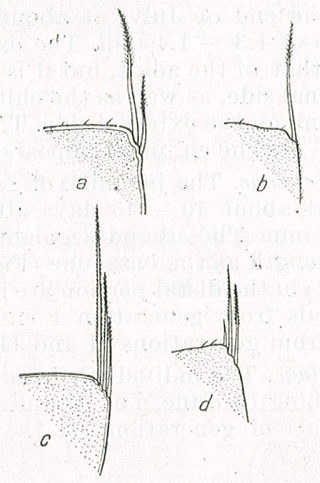

Fig. 5. — *L. hypnorum* — aspects of the variation of thorns number in the male exopodite of pleopod I.

of pleopod II (the gonopod) is of a relatively constant shape in the adults, presenting only stage variations. The exopodites of uropods have a positive allometric growth, and they also present only stage variations.

Trichoniscus pusillus is a species with a very large distribution area and presents many difficulties in determination [8], [10]. Four subspecies have been described, but after some revisions [4], [8] only 3 subspecies were accepted. In this paper we used only the binominal name, because after studying the ontogenetic development we came to the conclusion that a new revision was necessary. First, one must know the ontogenetic development of all populations of the described subspecies.

Larval stage. The larvae of *T. pusillus* have 1 — 1.2 mm. in size at hatching. With these larvae the tegument is slightly pigmented, too.The pereion 7 is incompletely developed, and the pereiopod 7 is absent. The antennary flagellum is formed of 3 segments, the eyes are composed of 3 ommatidia and the length ratio ex. / en. of uropods is 1.2 — 1.3. In *T. pusillus* larvae, these features are the same with those of the adult, while in *L. hypnorum* they undergo stage variations.

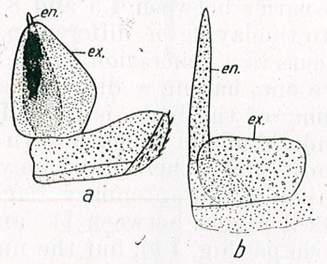

Fig. 6. — *Trichoniscus pusillus — immature stage. a* — pleopod I; *b* — pleopod II.

Immature stage. The first immature individuals of generation appear at the end of July, at about 20 — 25 days after the hatching. Their size is of 1.3 — 1.4 mm. The exopodite of pleopod I has a similar shape with that of the adult, but it is lacking the row of subterminal hairs on the internal side, as well as the chitinous lobe, which in the adult pleopod is present on the external side. The second segment of en. pl.I is very short (Fig. 6 *a*). The en. pl. II appears as a dorso-ventrally flattened stalk.

Juvenile stage. The juveniles of generation I appear at the beginning of August, at about 40 — 45 days after the hatching, and their size is of 1.4 — 1.7 mm. The second segment of the en. pl. I is approximately of the same length as the basal one (Fig. 7 *b*). The borders of the en. pl. II (the gonopod) in the distal portion are revolved (Fig. 7 *c*). With *T. pusillus* the individuals from generation I enter the diapause also as juveniles, while those from generations II and III as immatures and larvae.

Adult stage. The individuals from generation I and II become adults at the beginning of June, i.e. 10 and 11 months after hatching. Among the individuals of generation III (hatched in September and being in

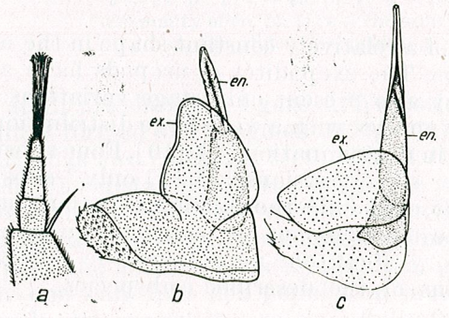

Fig. 7. — *T. pusillus — juvenile stage. a* — antenna; *b* — pl. I; *c* — pl. II.

diapause as larvae) we found very few animals and we noticed that their development after the diapause is greatly slowed down. It seems that they don't reach the adult stage, but this must be verified under laboratory conditions. The adult characters in *T. pusillus* are given in figure 8. We underline that with the 2 — 3-year-old male adults the external lobe of the ex. pl. I is prolonged by a fine chitinous fold, obliquely directed

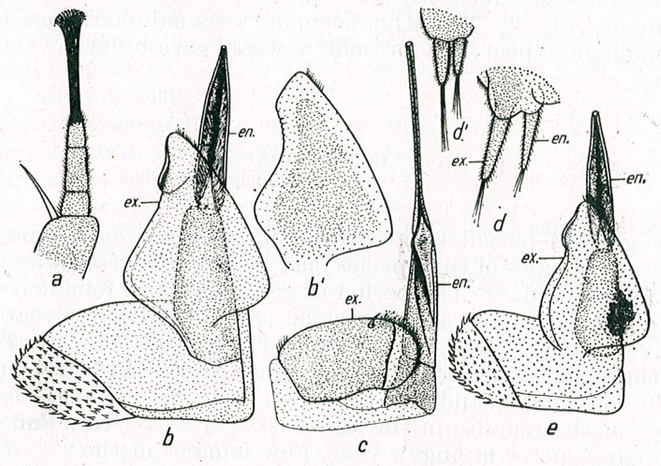

Fig. 8. — *T. pusillus — adult stage. a* — antenna; *b* — pl. I in 2 — 3-year-old adult male; *b'* — ex. pl. I pressed on the slide; *c* — pl. II; *d* — adult uropods; *d'* — larva uropods; *e* — pl. I in 1 — 2-year-old adult male.

toward the internal side (Fig. 8 *b*). If the exopodite is pressed on the slide the fold disappears (Fig. 8 *b'*). We gave this image too, because we found it in different works of systematics, but it is not a real representation

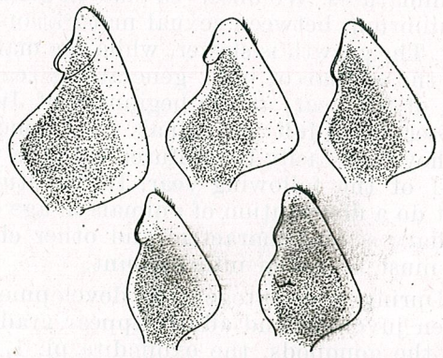

Fig. 9. — *T. pusillus.* Aspects of the variability of exopodite of pleopod I in adult males.

of the exopodite of pleopod I. In the young male adults the chitinous fold is absent, only the lobe on the lateral edge being present (Fig. 8 *e*). In *T. pusillus* there is also a clear difference in size between the adults

of the two generations. The adult males from generation I are of 1.8—2 mm., while those from generation II are of 1.4 — 1.6 mm. More difficult is the differentiation between the adult and juvenile individuals, for excepting the pleopods we have not found other features (antennary flagellum or uropods) with stage variations. Colour and size cannot be criteria of distinction between adults and juveniles. Beside stage variations, as phenotypic variations we observed a strong variability of the ex. pl. I shape in adult males (Fig. 9). The other secondary sexual characters have a simple morphologic aspect and present a weak variability.

CONCLUSIONS

The general period of gestation in *Ligidium hypnorum* (period in which gestant females of this species may be found) is from May till August, of about 90 — 100 days. The period of gestation for a female is of 40 — 50 days. The females in the age classes of 1 — 2 and 2 — 3 years have two consecutive layings a year, and those of 1 year, only one. In *Trichoniscus pusillus* the general period of gestation is from May till September, of about 130—140 days, and the gestation time for a female is of 30—35 days. Most of the females in the age classes of 2—3 years and 1—2 years have two consecutive layings a year. Few females of the 2 — 3 years class have three consecutive layings. The 0 — 1-year-old ones are laying once. With both species, the number of eggs layed by a female depends on size, age and range number of laying.

Sexual differentiation for the larva of generation I in *Ligidium hypnorum* starts after the 5th of August, and in *Trichoniscus pusillus* after the 25th of July. Sexual differentiation for the larvae of generation II occurs during the month of September. With both species, the individuals of generation I enter the diapause as juveniles, and those of generation II as inmatures. We observed that in generation II the diapause causes a disequilibrium between sexual maturation and growth in the next year's spring. The growth is slower, while the maturation processes are hastened. Thus, the animals of both generations reach the adult stage in the same period of the year, at the beginning of June. Evident differences of size are observed. Adult individuals from generation II are much smaller, so that they may easily be confused with the juvenile individuals from generation I of the following year. In the study of populations ecology, one cannot do a distribution of animals in age classes based only on their size. Secondary sexual characters and other characters having a stage variability must be taken into account.

During the ontogenetic development, the distinctive characters between juveniles and adults appear gradually. The first formed are the penis, the gonopods, the exopodite pl. I, and then the other characters are defined. In their biological cycle there are periods in which both adult and juvenile characters are present in the same individual. In the delimitation of adults from juveniles, this aspect as well as the whole aggregate of characters must be taken into account.

REFERENCES

1. Besse G., Juchault P., Legrand J.J., 1968 (1969), C.r. Séanc. Soc. Biol., **162,** *12,* 2196–2200.
2. Beyer R., 1957—1958, Wiss. Zt. Karl-Marx Univ. Leipzig, Reihe 2/3, 291—308.
3. Cherniaux-Cotton J., 1970, Bull. Soc. Zool. France, **95,** *3,* 565—594.
4. Flasarova M., 1962, Acta Soc. Zool. Bohemoslov., **24,** *2,* 110—116.
5. Juchault P., 1968, Bull. Soc. Zool. France, **93,** *3,* 459—465.
6. Juchault P., Legrand J.J., 1968, C.R. hebd. Séanc. Acad. Sci. Paris, **267D,** *23,* 2014—2018.
7. Juchault P., Besse G., Legrand J.J., Mocquard J.P., 1969, C. R. hebd. Séanc. Acad. Sci. Paris, **268D,** *21,* 2505—2607.
8. Legrand J.J., Strouhal H., Vandel A., 1950, Bull. Soc. Zool. France, **75,** *5—6,* 307 — 312.
9. Meinertz Th., 1951, Zool. Jb., **63.,** *1,* 1 — 24.
10. Racovitza E.G., 1908, Biospeologica, IX, Arch. Zool. Expér. Gén., (4), IX, 239 — 415.
11. Radu V., Tomescu N., 1971, Rev. roum. Biol. — Zool., **16,** *2,* 89 — 96.
12. Reidenbach J.M., 1966, C.R. hebd. Séanc. Acad. Sci. Paris, **262D,** *6,* 682 — 684.
13. Tomescu N., 1972, Rev. roum. Biol. — Zool., **17,** *1,* 31 — 39.
14. Vandel A., 1957, C.R. hebd. Séanc. Acad. Sci. Paris, **244,** 979 — 984.
15. — 1960, Ann. Speleol., **15,** *3,* 553 — 584.
16. Verhoeff K.W., 1917, Arch. Nat., **83,** *S,* 1 — 54.
17. — 1920, Zool. Anz., **60,** *S,* 169 — 189.

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LA MICROSCOPIE ÉLECTRONIQUE DES HISTONES DANS L'OOGENÈSE DE *CARASSIUS AURATUS* PAR RÉACTION DE L'ARGENT AMMONIACAL

PAR

MIRCEA IONESCU-VARO

The arginine rich histones could be seen in the electron microscop within the oocytes of *Carassius auratus* by means of the ammoniacal silver reaction following fixation in buffered formalin. The product of the reaction is seen as little electronic-opaque particles which are associated within the cytoplasm and the nuclei with r-ARN forming a ribosomal protein complex and within the follicular nuclei the heterochromatin ADN.

On a constaté ces derniers temps que les ribosomes des oocytes contiennent des protéines basiques du genre des histones [3] [4] [5], [6], qui ont pu être détectées par des méthodes convergentes histochimiques [11]. Il existe d'ailleurs une série de contributions précieuses à l'analyse des histones attachés à l'ARN des nucléoles et du noyau [2], de sorte que nous pouvons affirmer à juste raison que le même genre de protéines sont attachées à l'ADN et à l'ARN. Dans le présent article nous nous proposons de mettre en évidence par des moyens cytochimiques ces protéines dans les oocytes du caras.

MATÉRIEL ET MÉTHODES.

De petits morceaux d'ovaires du poisson ont été fixés dans 10% paraformaldéhyde avec 2% acétate de sodium ou dans 3% glutaraldéhyde dans un tampon de cacodylate 0,1 M pH 7,4. Ils ont été lavés ensuite pendant une heure dans de l'eau distillée et traités à l'argent ammoniacal pendant 15 min, sous agitation permanente. L'argent ammoniacal est préparé à partir d'une solution 10% d'azotate d'argent versée goutte à

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goutte sur de l'hydroxyde d'ammonium concentré, en l'agitant aussi longtemps qu'un faible trouble persiste. Après le traitement à l'argent ammoniacal, on lave en changeant l'eau distillée environ dix fois et on réduit dans du formol 3% pendant environ 5 min. Les petits morceaux sont lavés à l'eau distillée et déshydratés à l'aide de l'alcool éthylique en concentrations criossantes, propylène oxyde et sont finalement inclus dans Epon 812. Les sections ont été faites avec des couteaux de verre à l'ultrotome LKB et après une double coloration avec du citrate de plomb-acétate d'uranyle, elles ont été examinées au microscope électronique Hitachi de l'Institut Dr.I. Cantacuzino.

RÉSULTATS

Nous avons analysé les oocytes surtout pendant leur petite croissance, avant la formation des vacuoles corticales. Au microscope photonique, tant sur les sections à paraffine que sur celles fraîchement coupées au cryotome, on observe que ces petits oocytes présentent une forte réaction aussi bien dans leur cytoplasme que dans les quelques grands nucléoles. Pendant ce stade se forme la « membrana radiata » à partir des prolongements du plasmolemme oocytaire et des cellules folliculaires entre lesquelles est déposé un matériel glycoprotéique.

Chez les très jeunes oocytes où la synthèse des extra-copies du r-ADN nucléolaire est encore en cours, les images obtenues au microscope électronique montrent une densité assez faible des particules de l'argent réduit dans le cytoplasme. On sait [8] [9] [10] que dans les stades très jeunes de l'oogenèse chez *Xenopus laevis* on ne trouve pas de ribosomes typiques et que le cytoplasme est peu actif dans la synthèse des protéines. Par contre, la basophilie est produite par un matériel fibrillaire qui correspond à des précurseurs ribosomiques qui vont se condenser progressivement en donnant naissance aux ribosomes seulement quand les oocytes auront atteint leur taille maximum. Il est fort posssible que dans notre cas également, lorsque le cytoplasme et le noyau montrent une faible condensation de ribonucléoprotéines, ce fait soit dû justement au début de la formation des ribosomes. On peut admettre que ceux-ci sont déjà formés dans les nucléoles, car la dimension des particules d'argent réduit est la même que dans la cytoplasme et on peut même surprendre leur migration par les pores du noyau. Au cours de la grande croissance, les oocytes ont des ribosomes abondants autant parmi les vacuoles que parmi les granules des vitellus formés. La méthode de l'argent ammoniacal permet aussi de détecter les protéines riches en histones des mitochondries où la reaction est produite par quelques petits granules de 40 Ä. En échange, les noyaux des cellules présentent une forte réaction due aux histones qui forment un complexe avec l'ADN chromosomial de l'hétérochromatine. Dans ce cas les particules sont beaucoup plus grandes, atteignant environ 200 Ä et pouvant former des agrégats de 500 Ä. Les ribosomes des cellules folliculaires à ergastoplasme donnent une réaction positive pour les protéines riches en arginine.

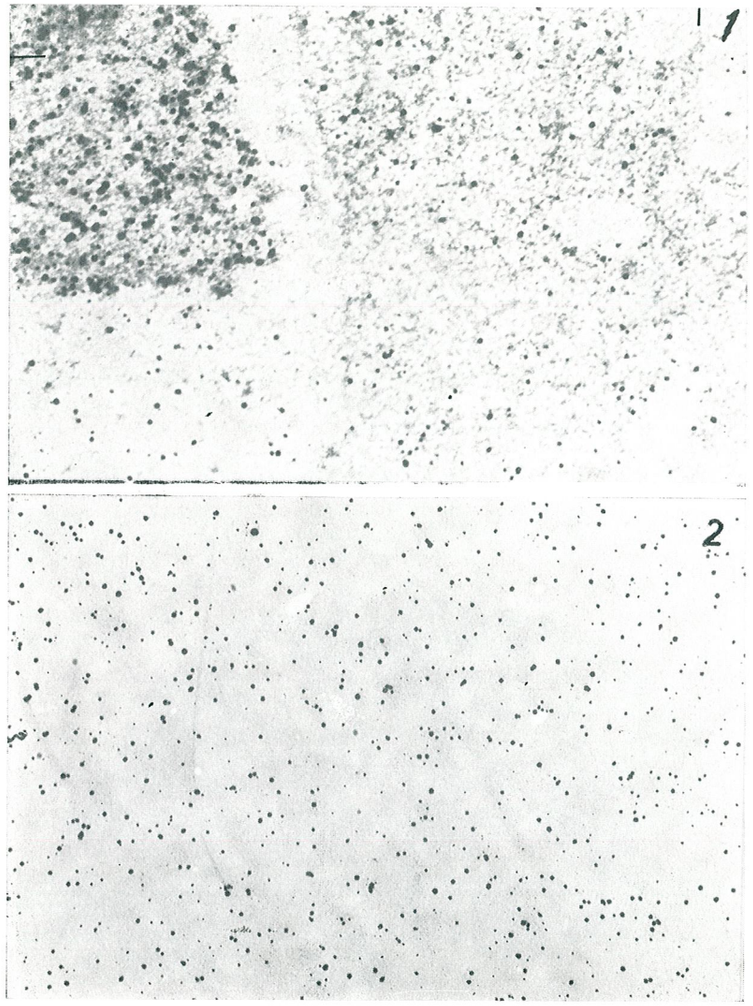

Fig. 1. — Une partie du noyau. nl, nucléole ; m, membrane nucléaire (× 20 000). Petites graines noires indiquant les protéines riches en arginine.

Fig. 2. — Cytoplasme de l'oocyte plein de graines d'argent (× 20 000).

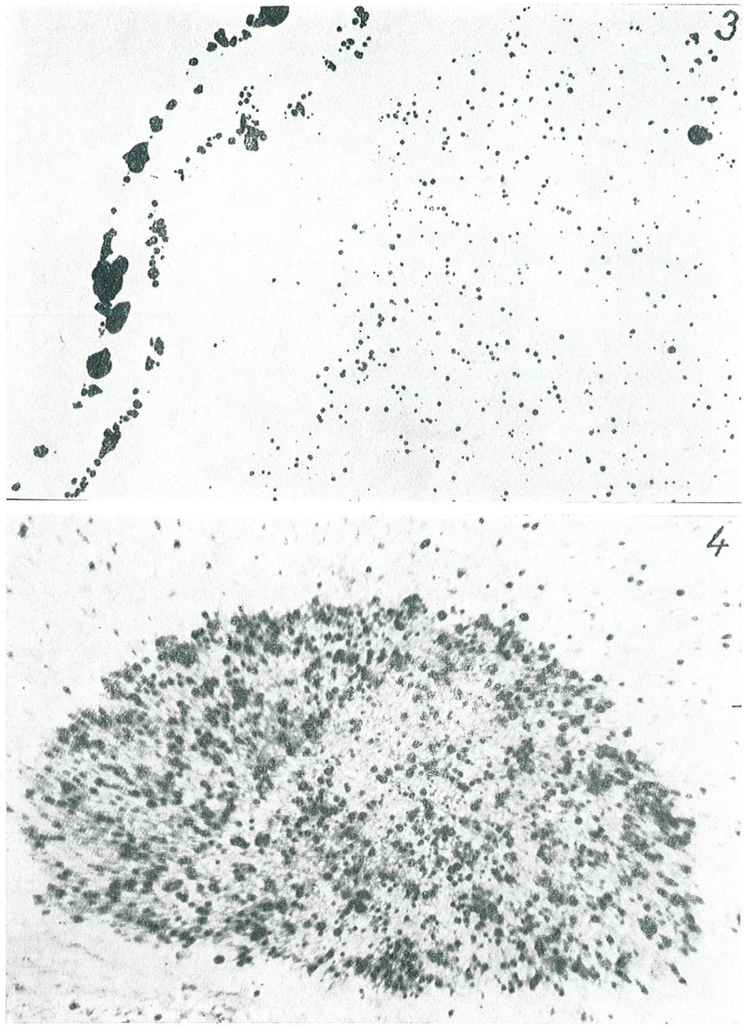

Fig. 3. — Noyau de cellule folliculaire (× 20 000).

Fig. 4. — Nucléole de la vésicule germinative (× 20 000).

La méthode de l'argent ammoniacal après fixation dans une aldéhyde tamponnée, introduite dans la cytochimie par Black et Ansley [1], permet de voir les histones au microscope photonique. Les histones riches en arginine prennent une couleur d'un brun bleuâtre foncé, tandis que les histones riches en lysine apparaissent en jaune. Cette méthode a été appliquée aux études électronomicroscopique par Varo [11] et par MacRae et Meetz [7]. En électronomicroscopie la réaction positive est produite par de petites particules sphériques ayant parfois un contour faiblement irrégulier, correspondant à la couleur brune-noire de la microscopie photonique. Cette couleur brune-noire est produite par les groupes guanidiniques de l'arginine. MacRae et Meetz [7] interprètent cette réaction comme étant produite par « l'interaction spécifique de l'argent avec les centres réactifs de l'arginine des protéines à riche contenu d'arginine ». Il est possible que la réaction soit produite aussi par des histones qui sont synthétisés ou réarrangés dans divers loci des cellules.

Des considérations présentées il ressort que, dès les très jeunes stades, on trouve dans les nucléoles des oocytes une quantité de protéines basiques riches en arginine associée à l'ARN nucléolaire. Ces complexes de ribonucléoprotéines passent dans le cytoplasme, qui s'enrichit ainsi progressivement en ribosomes, à mesure que se produit la grande croissance de la période de vitellogenèse. Les particules sont disséminées rapidement et uniformément car on les rencontre même dans les microvilli de la membrana radiata. Nous ne pourrons pas affirmer qu'il existe une région préférentielle dans les oocytes où s'accumulent les ribosomes ou les protéines basiques qui leur sont associés. Il y a toutefois des cas où la quantité de ribosomes autour du noyau est plus grande que dans le reste du cytoplasme.

BIBLIOGRAPHIE.

1. Black M.M., Ansley H.R., 1966, J. Histochem. Cytochem., **14**, 177—181.
2. Busch H. Smetana K., 1971, *The Nucleolus*, Acad. Press, New-Vork.
3. Davenpot R., Davenport J.C., 1965, J. Cell. Biol., **25**, 319—325.
4. — 1965, Exp. Cell. Res., **39**, 74—89.
5. — 1966, Exp. Cell. Res., **42**, 429—434.
6. Davenport R., 1967, Exp. Cell. Res. **47**, 397—402.
7. Macrae Edith K., Meetz D.G., 1970, J. Cell. Biol., **45**, 235—245.
8. Thomas C., 1967, Arch. Biol., **78**, 347—369.
9. — 1969, J. Embryol., exper. Morphol., **21**, 165—176.
10. — 1969, Arch. intern. Physiol. Bioch., **77**,
11. Varo M.V.I., Vlad Marcela, 1971, Z. wiss. Zool., **183**, 135—155.

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QUANTITATIVE VARIATIONS OF ADRENALINE AND NORADRENALINE IN THE ADRENAL GLAND OF *PHALACROCORAX CARBO* L. DURING ANNUAL CYCLE PHASES

BY

ANCA PETRESCU-RAIANU

The relative proportion of adrenaline and noradrenaline in the adrenal chromaffin tissue was estimated by counting differentially stained cells with Mallory staining applied to preparations previously fixed in bichromate-containing mixtures. Determinations were carried out during March, May and October. Three distinct kinds of cells were found : adrenaline-containing cells, noradrenaline-containing cells and intermediate ones. Major amine for both sexes in any given stage was adrenaline, which reached the highest level in March and the lowest one in May. Noradrenaline, however, was relatively constant in proportion. The number of intermediate cells was quite variable and might be used as an index for the physiological state of adrenal chromaffin tissue.

The quantitative ratio of the two catecholamines was approached in several studies. Coupland et al. [4] pointed out that their relative proportion in normal adults is species specific. Nevertheless, differences were found in the adrenaline/noradrenaline ratio during embryonic development as compared to that recorded for adult individuals. Several investigators reported that during early developmental stages noradrenaline is almost the only amine to be found in the adrenal gland. Adrenaline becomes apparent only in later stages and increases until the species characteristic level is reached [22], [24], [26], [33].

In birds, the relative proportion of the two catecholamines shows large variations. In several species, noradrenaline is practically the only catecholamine in the adrenal gland, while in other ones the reverse situation occurs [1], [8], [9], [13], [23]. Ghosh and Ghosh [9] suggested a relationship between phylogenetic evolution and the adrenaline/noradre-

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naline ratio, with primitive forms (*Gallus*, *Anas*) synthesizing preponderantly noradrenaline and Passeriformes, a recently evolved group, showing a higher level of adrenaline. The present study is concerned with the relative proportion of the two amines in a more primitive bird species than those so far investigated and with its suspected variation during the annual cycle.

MATERIAL AND METHODS

Adrenal glands of adult male and female individuals of *Phalacrocorax carbo* were removed during March, May and October, corresponding to definite stages of the sexual cycle, i.e., March to the mating period, May to egg laying and hatching and October to sexual repose. The number of examined individuals is given in the table.

To demonstrate selectively the catecholamines use was made of following fixation mixtures: Orth, potassium chromate-potassium bichromate at pH 5.6 and potassium bichromate-formaldehyde. When the potassium chromate-potassium bichromate mixture was used, a 24 hrs fixation was followed by a post-fixation in 10% formaldehyde. The material was freeze-sectioned in some cases, while another series was included in paraffin (fast inclusion), sectioned and stained in a mixture containing aniline blue, orange G and phosphomolybdic acid, according to Mallory.

Adrenaline and noradrenaline amounts were quantitatively estimated by counting differentially stained cells with Mallory staining applied to both chromaffin islets subcapsulary in location and to those located in the central zone of the gland. 400—600 cells in each zone were recorded for any individual and the results were expressed as per cent values.

RESULTS

In *Phalacrocorax carbo*, adrenal chromaffin cells appear as groups of different size, randomly distributed among interrenal cords.

By means of the chromaffin reaction of Hillarp and Hökfelt we failed to distinguish without doubt the two types of chromaffin cells (Fig. 7). However, this became possible with Mallory staining as applied to material previously fixed in bichromate-containing solutions. The preparations allowed a clear-cut distinction between adrenaline-containing cells which stained in blue, the noradrenaline-containing ones which stained in yellow and a third cell type, intermediate cells. These latter are different in appearance: some are stained in mustard yellow, while others display within the cytoplasm of the same cell, yellow zones alternating with blue ones (Fig. 3).

In the central part of the gland, all chromaffin islets are mixed, containing adrenaline cells as well as noradrenaline and intermediate cells (Figs 2 and 6). Though most often mixed, peripheral islets also occur with one major kind of cells (Figs 1 and 4).

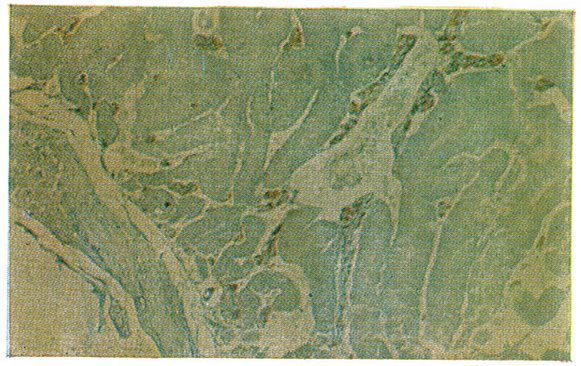

Fig. 1. — A part of a section through the adrenal gland of *Phalacrocorax carbo*, with chromaffin islets comprising, in the peripheral zone, mainly adrenaline-containing cells (potassium chromate and potassium bichromate; Mallory).

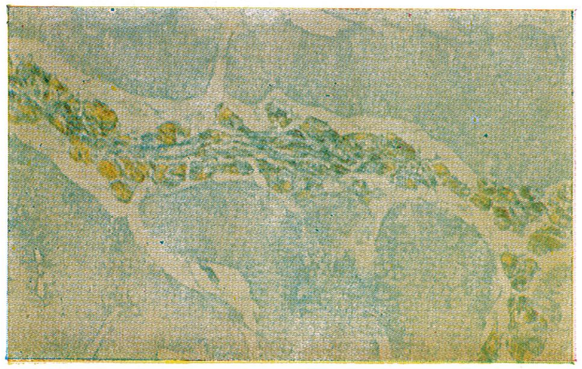

Fig. 2. — Chromaffin islet in the central zone containing adrenaline cells (blue), noradrenaline cells (yellow) and intermediate cells (mustard yellow). Note the location of noradrenaline cells at the periphery of the islet (potassium chromate-potassium bichromate; Mallory).

Fig. 3. — Chromaffin islet built up of the three different kinds of cells (potassium chromate-potassium bichromate; Mallory).

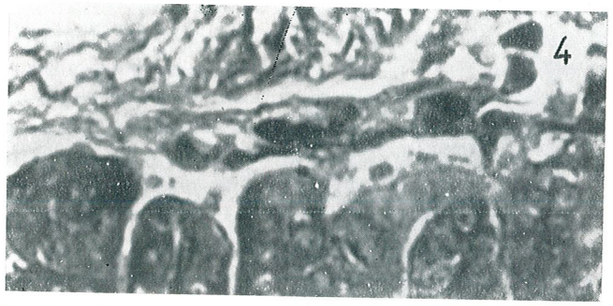

Fig. 4. — Peripheral small chromaffin islet, built up only of adrenaline-containing cells (potassium chromate-potassium bichromate ; Mallory).

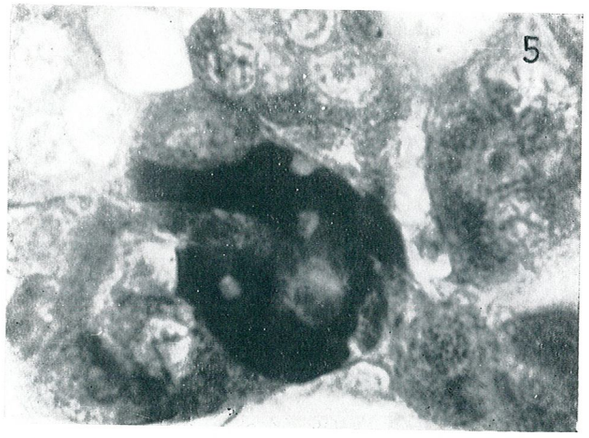

Fig. 5. — Adrenaline-containing cells with an apparent vacuolization (potassium chromate-potassium bichromate ; Mallory).

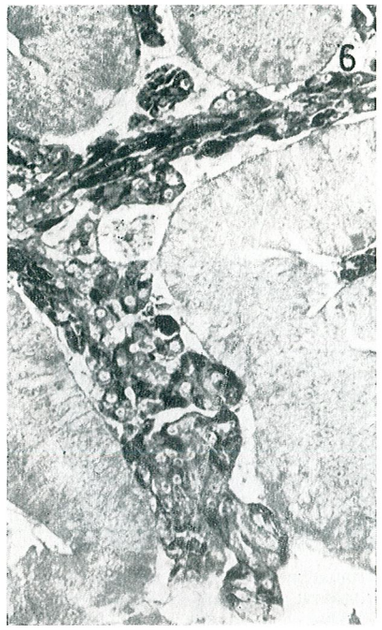

Fig. 6. — Central chromaffin islet (potassium chromate-potassium bichromate ; Mallory).

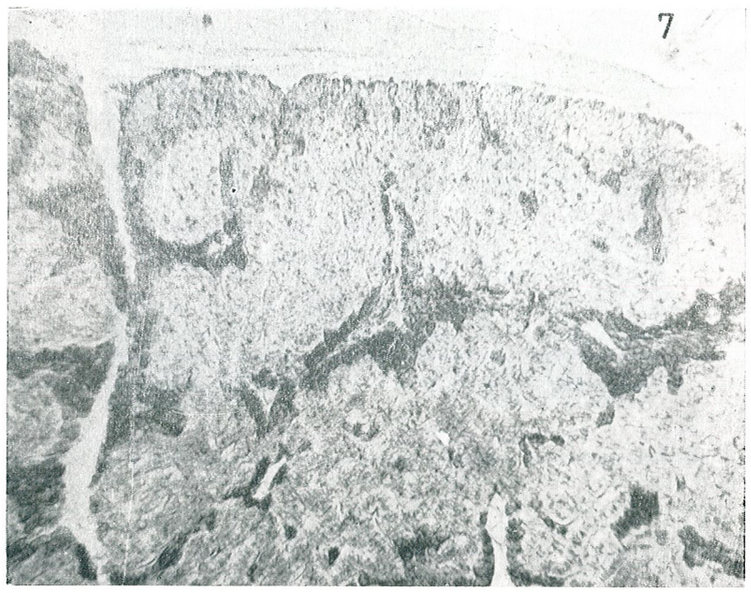

Fig. 7. — The distribution of chromaffin islets in a sector of the gland (chromaffin reaction according to Hillarp and Hökfelt).

Within an islet, noradrenaline cells are usually peripheral in location (Fig. 2). Several chromaffin cells show an apparent vacuolization of the cytoplasm, which occurs in each one of the three kinds of cells in the chromaffin tissue (Fig. 5).

In spite of individual variation of the relative volume of the three types of cells, several conclusions might be drawn. The data in the table indicate that in *Phalacrocorax carbo* the major amine in the adrenal gland is adrenaline, regardless of sex and annual cycle stage.

The occurrence of adrenaline-containing cells usually took values between 50 and 65 p.c. with some minor exceptions dependent upon the annual cycle phase. Furthermore, peripheral islets appear to contain a higher proportion of adrenaline as compared to those in the central zone of the gland, during any of the annual cycle phases so far investigated. An examination of the graph and table reveals a relative constancy of noradrenaline amounts which seem to be independent of the annual cycle phase, sex and even zone of the gland (Fig. 8 and table 1).

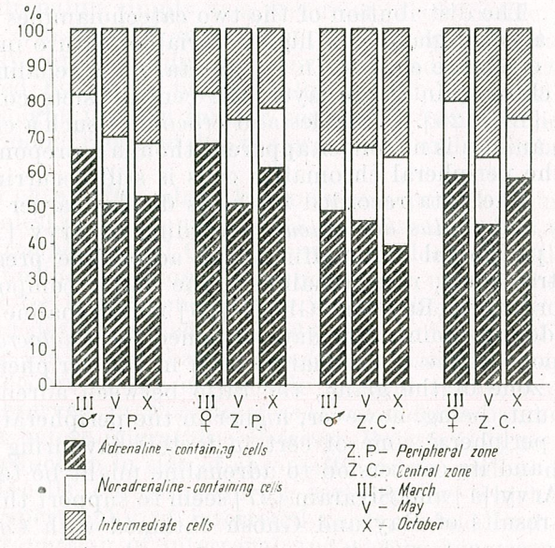

Fig. 8. — The number (in p.c. values) of adrenaline, noradrenaline and intermediate cells as a function of annual cycle stage, sex and zone of the gland.

The changes in adrenaline content seem to follow the same pattern for both sexes, as well as for different parts of the gland, though the graph corresponding to the central zone in male displays a rather different aspect. The higher adrenaline level was found in March, while in May the lowest one was recorded. In October, intermediate amounts were found to occur.

Table 1

The number of adrenaline-containing cells (A-cells), noradrenaline-containing cells (Na—cells) and intermediate cells (I-cells) in the adrenal glands of *Phalacrocorax carbo*, as a function of annual cycle stage, sex and zone of the gland (in p.c. values)

Stage	Sex	No. of birds	Peripheral zone			Central zone		
			A-cells	NA-cells	I-cells	A-cells	NA-cells	I-cells
March		5	65.64	16.14	18.22	48.82	16.68	34.24
		12	68.04	13.85	18.08	59.81	21.10	19.89
May		5	50.56	18.96	30.46	45.66	21.22	33.12
		5	51.22	23.94	24.82	44.82	19.08	36.06
October		5	52.66	15.54	31.90	38.62	24.74	36.62
		10	60.32	17.23	22.24	57.63	16.82	25.48

DISCUSSION

The distribution of the two catecholamines among different parts of the adrenal gland is a highly variable feature in most representatives of all vertebrate classes. In Squamata, noradrenaline is confined to the dorsal chromaffin tissue layer, the central islets containing adrenaline only [5], [15], [25]. In turtles and crocodiles such a clear-cut separation of the two amines is no longer apparent though a preponderance of noradrenaline in the peripheral chromaffin cells is still occurring in crocodiles [6], [7].

The data recorded for birds display larger variations. In *Anas boschas* and *Gallus domesticus*, according to Arvy [1] and Sivaram [27], in the peripheral chromaffin tissue adrenaline predominates, while in the central islets, noradrenaline is the major component. In *Columba livia*, according to Ray and Ghosh [23] noradrenaline is the peripherally preponderant amine. Our data obtained for *Phalacrocorax carbo* indicate the preponderance of adrenaline both in the peripheral islets and in the central zone of the gland, the ratio between adrenaline and noradrenaline amounts being, however, higher in the peripheral zone. The occurrence in the peripheral zone of certain factors favouring noradrenaline methylation and its conversion to adrenaline might be tentatively inferred. Data by Arvy [1] and Sivaram [27] seem to support this interpretation, though the results of Ray and Ghosh obtained with *Columba livia* [23] are in disagreement with it.

The species-dependent variations in the relative proportion of the two catecholamines in adrenal gland was not yet satisfactorily explained. The animal behaviour was expected to account for the different catecholamine proportion, aggressive animals displaying higher levels of noradrenaline [10]. Ghosh and Ghosh [9], on the other hand, suggested a relationship between the proportion of the two catecholamines and the position of the species within phylogenetic lineage : representatives of the genera *Anas* and *Gallus* synthesize mainly noradrenaline, while Passeriformes, as a recently evolved group, show a higher content of adrenaline.

Our data do not support either of the two hypotheses. *Phalacrocorax carbo*, though a predatory, carnivorous (ichthyophage) bird, phylogenetically primitive, shows a much higher content of adrenaline as compared to noradrenaline amounts. Based upon the individual variation in *Phalacrocorax carbo* reported above, as well as additional data reported by other authors, we assume that the proportion of the two catecholamines is not in the least fixed and it does not seem to depend upon phylogenetic position, displaying rather large variations corresponding to the requirements of the organism in certain physiological states.

According to the data recorded for Squamata as well as for certain mammals [4], [5], [7], [14], [28], [29], chromaffin cells seem to be not uniformly able to synthesize both catecholamines i.e. to methylate noradrenaline to adrenaline.

Picard, Vitry et al. suggested that adrenaline-containing cells and noradrenaline-containing ones are the two morphologic appearances of chromaffin cells, ascribable each to a different stage of the secretory cycle [3], [20], [21], [31], [32]. This conclusion is based upon the occurrence in chromaffin tissue of cells with intermediate features between adrenaline and noradrenaline cells, which we observed in *Phalacrocorax carbo*, though other authors did not note them. However, it does not seem reasonable to consider noradrenaline simply as a precursor of adrenaline, as it was detected in adrenal effluent and, in addition, during early developmental stages it is the only catecholamine to occur in the adrenal gland. The vacuolization observed by us in noradrenaline-containing cells of adult *Phalacrocorax carbo* might provide further support to consider noradrenaline as a true hormone, required by the organism under certain conditions.

Seasonal variation of chromaffin adrenal tissue was reported mainly in mammals. Although Zalesky [34] found a lack of seasonal variation in adrenal medulla of *Spermophilus* and Canguilhem and Bloch [2] failed to reveal a seasonal cycle of urine excretion of catecholamines, most authors assume an important part for adrenal medulla in hibernation of rodents [11], [18], [19], [30]. The data reported for birds are controversial as well. Lorenzen and Farner [16] show that in *Zonotrichia leucophrys gambelii* no seasonal change occurs in the chromaffin adrenal tissue, while John and George [12] observed in *Sturnus roseus* an enhancement of adrenaline production just before migration; Petrescu-Raianu [17] noted in *Phalacrocorax carbo* a reduction in size of chromaffin islets and a degeneration of a part of their cells, occurring during early spring, which in turn, together with an hypertrophy of interrenal cords, would lead to changes in the relative proportion of interrenal and chromaffin tissues.

The relative constancy of the number of noradrenaline cells, as well as the variation of the number of intermediate cells suggest that in different stages of the annual cycle the adrenaline requirements are variable. During May the lowest level of adrenaline was recorded in the adrenal gland of *Phalacrocorax carbo*. One might conclude that adrenaline was released from the gland, to fulfil certain needs of the organism. As during the same period the number of intermediate cells also reaches highest value, i.e. the conversion of noradrenaline to adrenaline proceeds at a high rate, the above assumption seems to be even more plausible. The number of

intermediate cells might, thus, represent an index for the physiological state of adrenal chromaffin tissue and for the adrenaline requirements of the organism.

The high level of adrenaline and the occurrence of a reduced number of intermediate cells during March indicate a low activity of the chromaffin tissue in this period, which seems to be related to the chromaffin tissue degeneration described by us previously [17]. Taking into account the reduction in the adrenaline amounts stored in the gland the increase in the number of intermediate cells during May, it seems reasonable to think that an activation of this tissue takes place due to the hormone requirements related to egg laying and hatching.

CONCLUSIONS

1. Adrenaline is the major amine in the adrenal chromaffin tissue of *Phalacrocorax carbo* in all phases investigated.

2. The distinct morphologic appearances of chromaffin cells correspond to different phases of a secretory cycle. It does not mean that noradrenaline is not an active hormone of the adrenal gland required for certain physiological states of the organism.

3. During the annual cycle a variation exists in the number of adrenaline-containing and intermediate cells. The noradrenaline amount is, however, relatively constant. This pattern suggests that intermediate cells represent an index of the physiological state of the adrenal chromaffin tissue and of the adrenaline requirements of the organism.

4. The high level of adrenaline and the small number of intermediate cells during the month of March indicate a low activity of the chromaffin tissue during this period. During May, however, a stimulation of this tissue occurs, as revealed both by the reduction in the amount of adrenaline stored in the chromaffin cells and by an increase in the number of intermediate cells.

REFERENCES

1. Arvy L., 1961, C.R. Soc. Biol., **155**, *1*, 69—71.
2. Canguilheh B., Bloch R., 1967, Arch. Sci. Physiol., **21**, *1*, 27—44.
3. Cotte C., Monneron A., Michel-Béchet M., Picard D., 1964, Bull. Assoc. Anat., **49**, 485—493.
4. Coupland R.E., Pyper A.S., Hopwood D., 1964, Nature, **201**, *4925*, 1240, 1242.
5. Gabe M., Martoja M., 1961, Arch. Anat. Micr. Morph. exp., **50**, *1*, 1—34.
6. — 1962, Arch. Anat. Micr. Morph. exp., **51**, 107—128.
7. Gabe M., Rancurel P., 1964, Arch. Anat. Micr. Morph. exp., **53**, *3*, 225—240.
8. Ghosh I., Ghosh A., 1962, Proc. Zool. Soc., **15**, 135—149.
9. — 1964, Z. Biol., **114**, *5*, 400—404.
10. Goodall M.C., 1951, Acta physiol. scand., **24**, suppl. 85.
11. Hoffman R.A., Hester R.J., 1965, Nature, **207**, 656—657.
12. John T.M., George J.C., 1968, Pavo Indian J. Ornithol., **5**, *1—2*, 47—51.

13. Lahiri P., Ghosh I., Ghosh A., 1967, Folia biol., **15**, *1*, 35—41.
14. Lanza G., 1962, Bull. Assoc. Anat., **112**, 322—326.
15. Lofts B., Phillips J.G., 1965, J. Endocrinol., **33**, *8*, 327—328.
16. Lorenzen L.C., Farner D.S., 1964, Gen. comp. Endocrinol., **4**, *3*, 253—263.
17. Petrescu—Raianu A., 1973, St. Cercet. Biol., Ser. Zool., **25**, *5*.
18. Petrovič V.M., Davidovič V., 1963, C.R. Soc. Biol., **157**, 1175—1177.
19. 1965, J. Physiol. (Paris), **57**, 678—679.
20. Picard D., Chambost G., Vitry G., 1953, C.R. Soc. Biol., **147**, 460—462.
21. Picard D., Vitry G., 1964, Arch. Biol., **75**, *3—4*, 453 — 496.
22. Picon R., 1964, Arch. Anat. Micr. Morph. exp., **53**, *4*, 343—356.
23. Ray I., Ghosh A., 1961, Acta histochem., **11**, 1—4.
24. Roffi J., 1968, Ann. Endocrinol., **29**, *3*, 277—300.
25. Saint—Girons H., 1963, Arch. Anat. Micr. Morph. exp., **52**, *1*, 1—51.
26. Shepperd D.M., West G.B., 1951, Brit. J. Pharmacol., **6**, 667—674.
27. Sivaram S., 1965, Can. J. Zool., **43**, *5*, 1021—1031.
28. Smollich A., 1967, Endokrinologie, **52**, *1—2*, 110—119.
29. — 1967, Z. Mikrosk. Anat. Forsch., **77**, *1*, 73—89.
30. Uuspää V.J., 1963, Ann. Med. exp. Fenn., **41**, 349—353.
31. Vitry G., 1964, Ann. Histochim., **9**, suppl., 1.
32. — 1967, Arch. Anat. Micr. Morph. exp., **56**, *1*, 31—42.
33. West G.B., Shepperd D.M., Hunter R.B., 1951, Lancet, **261**, 966—969.
34. Zalesky M., 1934, Anat. Record, **60**, 291—321.

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THE EFFECT OF THIOUREA AND UREA ON THE OXYGEN CONSUMPTION IN *ANODONTA CYGNEA*

BY

C. A. PICOŞ

The author studied the action of thiourea on the oxygen consumption in molluscs acclimated to 25° and 30°C, as well as the action of urea on the oxygen consumption in molluscs acclimated to 25°C.

Both substances were administered in water, in the same dose (1 g./l.) and over the same period of time. Oxygen determination in water was made according to Winkler's method.

The mean values of oxygen consumption recorded before, during and after cessation of treatment evidenced the following:

1. Under thiourea action the oxygen consumption increases by 83.40% in molluscs acclimated to 25°C and by 127.56% in molluscs acclimated to 30°C.
2. Under the action of urea the oxygen consumption in molluscs acclimated to 25°C decreases by 27.96%.
3. With both substances the oxygen consumption of molluscs tends to revert to the initial values 24 hours after the cessation of treatment.

The findings of our researches effected in recent years [3]—[6], [8] have drawn the attention on the particular importance presented by the investigation of the action of some thic derivatives (thiourea and methylthiouracil), commonly known in physiology under the name of antithyroids, on the energy metabolism of poikilothermic animals.

At least two facts, which we had made evident for the first time, aroused the interest for the study of this problem: 1) the species dependence of the metabolic action of thic derivatives; 2) the dependence of the same action on the temperature to which the animals have been acclimated.

The first fact resulted, on the one hand, from the experimental data obtained on molluscs [3], [6], and on the other, from those obtained

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on frogs [8]. Actually, under very different thermal conditions, treatment with a thioderivative produced an increase in the oxygen consumption of molluscs, whereas a decrease was recorded in frogs.

As to the second fact, we would first mention the results of some experiments [4] on fishes (*Carassius auratus gibelio* Bloch), which showed that the same thioderivative (methylthiouracil) administered in water, in equal dose (150 mg./l.) generated an increase in the oxygen consumption under the conditions of lower temperatures (7° — 10°C), and a decrease of the consumption when the temperatures were higher (20° — 23°C). Other investigators [2], [7] have not stated, however, a temperature dependence of the action of thioderivatives on the oxygen consumption in fish.

Also in respect of molluscs our previous investigations [3], [6] enabled us to find a certain dependence on temperature of the metabolic action of a thioderivative (thiourea) consisting in the fact that the metabolic effect of this substance is more pronounced under the conditions of low temperatures (+ 5°C) and less pronounced when the temperatures are higher (18° — 20°C).

As the results of experiments on fishes suggested the possibility that, at a certain thermic level, the reversal of the metabolic effect of thiourea should appear also in molluscs, we assumed the task to test the action of this substance at even higher temperatures. At the same time, to approach the problem of the inner action mechanisms of thiourea, we have tested, to a lesser degree, also the action of urea on the same physiological parameter.

MATERIAL AND METHOD

We have made our tests on six lots of molluscs (*Anodonta cygnea* L.) composed of 6—10 animals, of as far as possible equal sizes and having the same biologic antecedents. We carried out three experimental variants, using three lots in the first variant, two lots in the second, and one in the third. The three variants differed from one another through the different conditions under which the oxygen consumption of the animals was ascertained: 1) under the action of thiourea (1 g./l.) at 25°C; 2) under the action of the same substance (1 g./l.) at 30°C; 3) under the action of urea (1 g./l.) at 25°C.

In all the lots, the experiments were carried out in the same way which we describe hereafter. At least three days before beginning the experiments, the animals were acclimated to the corresponding temperature (25° or 30°C). For this purpose, the lots of animals were placed into cylindrical glass recipients of the same size and with the same amount of water from the tap (4 l.) which was ventilated by means of a vibrator air-pump. Then, the glass receptacles were immersed in the water of a large aquarium, maintained at the necessary temperature by means of a thermoregulation system. The water in the receptacle with animals was replaced daily only with the water of this particular aquarium in order to avoid whatever variations in temperature.

After the acclimation period, we carried out on different days, but at the same hours, 2—3 determinations of the oxygen consumption of the animals' lots maintained under standard conditions.

Over the next days the lots were treated with the substances the metabolic action of which was investigated (thiourea and urea). For this purpose, the same dose from the relative substance (1 g./l.) was administered daily in water, after its previous replacement. The oxygen

consumption of the animals was determined after 1, 2, 3, 4 and 5 days of action of the relative substance. Subsequently, determinations after the first and second day from the cessation of treatment were carried out.

To ascertain the oxygen consumption of the investigated animals' lots, we applied the confinement method using however respiring chambers of adequate capacities so that, at the end of the experimentation time (30 minutes), the oxygen contents of the limited volume of water never sank under 60% of the original value.

Measurement of water-dissolved oxygen was made according to Winkler's method. The oxygen consumption of molluscs was expressed in milliliters per kg. of active tissue and per hour.

For the first two experimental variants the values of oxygen consumption were joined, making up three groups of individual values (prior, concomitant and subsequent to thiourea treatment) which were then statistically processed.

For the third experimental variant, carried out in one lot (treated with urea) we calculated only the arithmetic mean of the three categories of data, as their small number was not suitable for a statistical processing.

RESULTS

In table 1 we give a synthetic presentation of the results obtained in the experiments carried out for the purpose of investigating the impact of thiourea (1 g./l.) on the oxygen consumption of molluscs acclimated to 25°C and to 30°C.

Table 1

Thiourea (1 g./l.) action on oxygen consumption of molluscs (*Anodonta cygnea*) acclimated to 25°C and 30°C

Experimental variant	Temperature (°C)	Mean values ($\bar{X}$) of O_2 consumption (ml./kg./h.) and their standard error (SE) ($\bar{X} \pm SE$)			P-values	
		Before treatment (A)	During treatment (B)	After treatment (C)	B—A	B—C
I	25	84.36±6.72 (6)*	154.72±7.82 (10)	70.50±1.10 (5)	P<0.00005	P<0.00005
II	30	62.98±6.91 (7)	143.32±5.59 (15)	82.35±9.80 (5)	P<0.00005	P<0.00005

* The figures in brackets show the number of tests carried out.

If we analyse the data from table 1, we find the following facts: first, before thiourea treatment, the oxygen consumption of the molluscs acclimated to 25°C is larger than that of those acclimated to 30°C. Indeed, if we compute the percentual difference between the relative mean values (84.36 and 62.98 ml. O_2/kg./h.) we find that, at 25°C, the oxygen consumption is by 33.94% higher than at 30°C.

Another finding is that under the conditions of both temperatures, thiourea treatment produces a considerable increase in the oxygen consumption of molluscs. In percentage this increase is of 83.40 % for the molluscs acclimated to 25°C and 127.56 % for those acclimated to 30°C. As a matter of fact, these important differences between the metabolic level prior to the treatment with thiourea and the one during the treatment, are reflected in the corresponding P values. Indeed, in both cases, $P_{B-A} < 0.0005$.

Finally, we find that even after the first 24 hours from the cessation of thiourea administration, the oxygen consumption considerably decreases

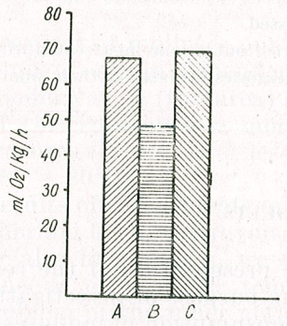

Fig. 1. — The oxygen consumption in molluscs (*Anodonta cygnea* L.) at 25°C: *A*, under standard conditions; *B*, during urea treatment; *C*, after cessation of treatment.

tending to approach the initial value (variant II) or even going below that value (variant I).

The results obtained in the third experimental variant, where we investigated the action of urea (1 g./l.) on the oxygen consumption of a lot of molluscs (composed of 8 animals) acclimated to 25°C are presented in figure 1.

The analysis of figure 1 enables us to find that the urea treatment (1 g./l.) produces a marked decrease in oxygen consumption of the molluscs acclimated to 25°C. Actually, if we compute the percentual difference between the average value of standard conditions (67.30 ml./kg./h.) oxygen consumption and that during urea treatment (48.48 ml./kg./h.) we find that the latter is by 27.96 % lower than the former.

With regard to the oxygen consumption after 1 or 2 days subsequent to the cessation of treatment, the relative average value (69.56 ml./kg./h.) is very close to the original average value (67.30 ml./kg./h.).

DISCUSSION

In contradiction with the hypothesis on which we started our investigations, no reversal of the impact of thiourea on the oxygen consumption of molluscs (*Anodonta cygnea*) occurred at high temperatures (25° and 30°C), as it happened in the case of fishes [4]. Indeed, the molluscs reacted to the thiourea (1 g./l.) treatment in the same way as those acclimated to 5°C [6], i.e. by an increase of their energy metabolism.

This identity of reactions under very different thermic conditions refers only to the direction of the metabolic effects of thiourea and not also to their amplitude, which differs depending on the acclimation temperature. Thus, whilst in the previous experiments [6] thiourea treatment given to molluscs acclimated to 5°C produced an increase of approximately 600 % in their oxygen consumption, the same treatment given to molluscs acclimated to considerably higher temperatures (25° and 30°C) produced much lesser hypermetabolic effects (83.40 % and 127.56 %, respectively).

The above stated facts, corroborated with the findings of similar research works carried out by us [3], enable us to assert that, broadly speaking, in molluscs the amplitude of hypermetabolic effects of thiourea decreases in parallel with the increase in the temperature of the setting.

Another fact which, by its seemingly paradoxical character, arouses a certain interest is that, under standard conditions, the oxygen consumption of the molluscs acclimated to a lower temperature (25°C) is by 33.94 % higher than the consumption of those acclimated to a higher temperature (30°C). Far from being paradoxical, the metabolic depression occurring at 30°C is wholly explicable by the fact that in the case of the relative animals this temperature goes beyond the border of applicability of the law which states that in poikilotherms the metabolism increases in parallel with the increase of temperature.

In support of this statement we would mention the fact that during the period of acclimation to 30°C some of the molluscs died, which means that this temperature is very close to their limit of thermic endurance.

After seeing that the broad outline of our investigation showed that thiourea, irrespective of temperature, produces an increase in the energy metabolism of molluscs, it was but natural that we directed our attention to the action mechanisms of this substance. Since the global energy metabolism reflects the cellular energy metabolism, we have to admit that thiourea exerts a direct stimulating action on the latter. We believe that since the molluscs have no thyroid gland, this primary action of thiourea is not affected by the intervention of other mechanisms which have their starting point in this gland as is, probably, the case with higher animals where — as it is known — thiourea produces a decrease in the energy metabolism.

The presence of sulphur in the thiourea molecule seems to justify the hypothesis according to which this substance stimulates the tissue respiration since — as known — sulfhydryl groups (— SH) play an important role in the enzymatic mechanisms of the respiratory chain [9].

An indirect proof supporting the fact that the sulphur is responsible for the hypermetabolic action of thiourea in molluscs, is supplied by the results of our experiments in connection with the impact of urea on the oxygen consumption of these animals. Indeed, urea which has an almost identical chemical structure with thiourea, except for the sulphur which is absent, no longer produced an increase in energy metabolism, evidencing — on the contrary — a decrease of the latter.

An at least partial explanation of the hypometabolic effect of urea was offered by the results of investigations carried out by Dita Cotariu [1] who found that in the striated musculature of vertebrates this substance

considerably reduces the activity of malic dehydrogenase which is an enzyme playing an important role in the oxidative metabolism.

This interesting finding suggests the idea that the hypermetabolic action of thiourea may be due — at least in part — to the stimulation by this thioderivative of the activity of the same enzyme.

CONCLUSIONS

1. In the molluscs investigated by us, acclimated to two different temperatures (25° and 30°C), the treatment with thiourea (1 g./l.) produced a considerable increase in their oxygen consumption: +83.40 % at 25°C and +127.56 % at 30°C.

2. With the same species of molluscs acclimated to 25°C, urea, administered in the same way and in equal dose (1 g./l.) determined a decrease in the oxygen consumption (−27.96 %).

3. With both substances, the oxygen consumption of molluscs tends to revert to the initial values 24 hours after cessation of treatment.

REFERENCES

1. Cotariu Dita, 1971, St. Cerc. Biol. Seria Zoologie, **23**, *3*, 249—254.
2. Gábos Márta, Pora E.A., Rău Letiția, 1973, St. Cerc. Biol. Seria Zoologie, **25**, *1*, 39—43.
3. Picoş C.A., Cucerzan M., 1967, Rev. roum. Biol. Zool., **12**, *3*, 195—201.
4. Picoş C.A., Schmidt Dumbrăviţa, 1968, St. Cerc. Biol. Seria Zoologie, **20**, *1*, 49—56.
5. Picoş C.A., Schmidt Dumbrăviţa, Popovici Elena, 1969, Zschr., vergl. Physiol., **63**, 146—150.
6. Picoş C.A., 1972, Rev. roum. Biol. Zool., **17**, *1*, 55—62.
7. Pora A.E., Roşca I.D., Wittenberger C., Stoicovici F., 1955, Bul. Inst. Cerc. pisc., **14**, *1*, 23—38.
8. Schmidt Dumbrăviţa, Picoş C.A., 1971, St. Cerc. Biol. Seria Zoologie, **23**, *4*, 331—337.
9. Torchinskii Iu. M., 1971, *Sulfghidrilnye i disulfidnye gruppy belkov*, Izd. Nauka, Moscow.

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CERTAIN METABOLIC ASPECTS OF THE CAROTENO-LIPID COMPLEX DURING EMBRYOGENESIS IN *LEPTINOTARSA DECEMLINEATA* SAY

BY

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The authors have followed the metabolism of the caroteno-lipid complex in the egg's vitellus of *Leptinotarsa decemlineata* Say. They have observed that during embryogenesis this complex is more active than the other lipid complexes.

Cheesman et al. [3], in their work entitled *Carotenoproteins in invertebrates*, have shown that besides real carotenoproteins — resulting from a stoichiometric association of carotenoids and proteins — the possibility arises to make lipoproteic combinations in which the prosthetic-lipid group should contain carotenoids in a non stoichiometric amount. In this case, the prosthetic group would not present any specific interaction with the protein, while the carotenoid would include a number of different molecular forms.

As a matter of fact, the structure of these complexes raise the problem of their possible role in the animal's life.

Certain works touching upon this problem contend that in eggs of invertebrates caroteno-proteins play the role of vitamin A. Currently, however, the presence of this vitamin has been spotted in the hemolymph of certain insects [6]. On the other hand, the association between carotenoids and reserve lipoproteins makes for the stabilization of the structure of lipid layers of internal and external cellular membranes with significant repercussions, as in the case of ovum maturation, when such membranes become permeable, transferring critical but necessary material to produce the egg [3].

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Our group has attempted to find out whether the caroteno-lipoproteic complex in the vitellus of *Leptinotarsa decemlineata* Say is able to support changes during the embryonic development.

MATERIAL AND METHOD

Work was conducted on eggs freshly brought from the field. After having been selected according to the colour they took at various stages of their embryonic development [4], the eggs were washed in water running through a sieve with pores of 0.143 mm. in diameter, with a view to removing impurities. The remaining water was carried away by a rapid flow of absolute ethanol. The eggs were then dried on filter paper, each time for the same interval. For analysis, out of the material thus prepared, the required amount was weighed up.

In this material the following substances were measured : total lipids [1], free and esterified total cholesterol [10], glycerides [7], phospholipids [8], free and esterified total fatty acids [1] of eggs freshly laid (yellow eggs), of eggs already in the third stage of their embryonic development (vermiform stage — red eggs) and of brown eggs showing the larva to be in the fifth stage of its development, when the head is joined to the cephalic region and when in the thoracic region rudimentary wings begin to emerge. At the same time, out of this material, carotenoids were exhaustively extracted with acetone. The extracts were subjected to the same analyses as the egg itself. In addition, pigments making up the carotenoid total extract underwent chromatographic separation on an activated Al_2O_3 column. Before being chromatographed the extract was transferred from acetone to lightpetroleum, dried on anhydrous SO_4Na_2 and photomeasured at 450 mμ and 20°, in order to calculate the amount of carotenoids [2], by using a Beckman DB spectrophotometer.

The control of the stage of embryonic development was made both by histologic means and by separating pigments on column chromatography, the emergence of certain oxygenated forms indicating a particular embryonic stage [4].

Since from the extract of total carotenoids different pigment forms were isolated without any prior separation from the lipid with which it was combined, we proceeded to check this complex by IR recording (Unicam SP 200).

RESULTS AND DISCUSSIONS

There are very few studies concerning lipid metabolism in insects and still fewer to approach the embryonic period. This is the reason why we have not been able to make comparisons. Nevertheless, analyses done on total lipids in the egg of *Leptinotarsa* virtually reveal that the development of embryogenesis follows, during the same period [9], almost the same way as that pointed to in literature for lipid metabolism in insects.

It should be recalled that *Doriphorus* consumes almost 12 % of the total lipids over the period of its embryonic development (Fig. 1*a*), which in comparison with the findings recorded in literature for other insects [9] shows a smaller consumption of lipids. It may be said that *Doriphorus* — an insect feeding especially on glucides — has as its main and direct source of energy glucides rather than lipids.

But the thing we are particularly interested in is the evidence that lipids combined with carotenoids in the big caroteno-lipo-proteic complex shows the same metabolic development as does the whole egg.

Out of the total lipids, as much as 17 % are consumed from the moment of egg laying to the moment of almost complete formation of the larvae. This observation enables us to infer that lipids combined with carotenoids are more reactive than lipids associated to or combined with other molecular forms (Fig. 1*b*).

Total fatty acids, particularly those in the caroteno-lipid complex, confirm the data found for total lipids (Fig. 1*a* and 1*b*). Their disappear-

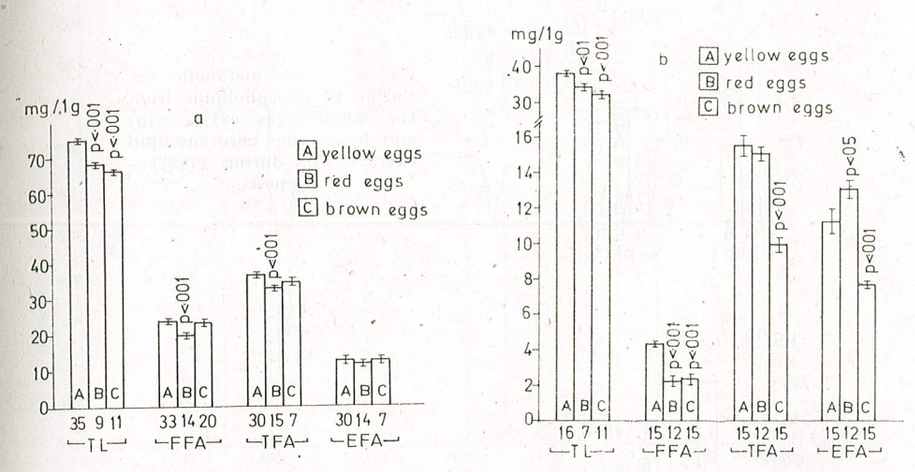

Fig. 1. — *a*, The metabolic variation of total lipids (TL), free fatty acids (FFA), total fatty acids (TFA) and esterified fatty acids (EFA) during embryogenesis from the whole eggs extract ; *b*, the metabolic variation of total lipids (TL), free fatty acids (FFA), total fatty acids (TFA) and esterified fatty acids (EFA) during embryogenesis from the caroteno-lipid extract.

ance during the time of complete organization of the embryo (red egg-brown egg period) coincides with the sharp decrease of phospholipids (Fig. 2) and of carotenoids (Fig. 3), revealing the active participation of both partners — lipids and carotenoids — in this process.

Indeed, IR recording of various pigment forms during the many stages of embryonic development points to their clear participation. Particularly noteworthy is the fact that during this evolution there occurs a modification in their spatial conformation. For instance, in yellow eggs all the pigments get the "cis" form (band at 1380 cm.$^{-1}$ assigned to the deformation of the methyl group vibration on a "cis"-double bond in the aliphatic system) [5], in the meantime some of them turning into the "cis"-"trans" form (band near 965 cm.$^{-1}$) [5]. Thus, the pigment we have spotted as being monadoxanthin is actually "cis" in the freshly laid egg, staying as such up to the vermiform stage of the larva (red egg) when "cis-trans" clearly appears in the 4th larval stage (when the three body regions have been formed). At the same time, in the freshly laid egg this pigment presents a certain esterification (stretching frequency of the unconjugated ester carbonyl function near 1735 cm.$^{-1}$) [5] as well as an OH^- amount, which in the red egg stage probably grows along with the development of a more intense esterification (Figs 4 and 5).

Finally, in the 5th embryonic stage (brown egg — the larva's head is joined to the cephalic region while in the thoracic region rudimentary wings begin to emerge), the molecule does no longer comprise OH^-, esterification is very low, and "cis-trans" conformation is clearly marked (Fig. 6).

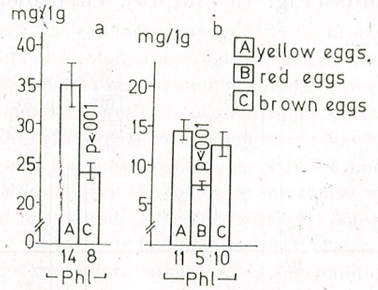

Fig. 2. — The metabolic variation of phospholipids from the whole eggs extract (*a*) and from the caroteno-lipid extract (*b*), during embryogenesis.

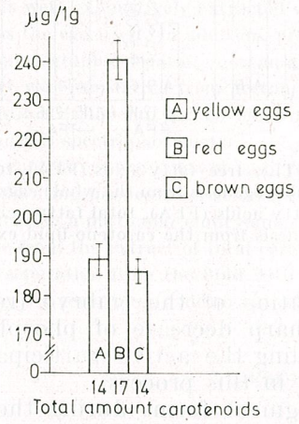

Fig. 3. — The carotenoid variation during embryogenesis.

The pigment dominating quantitatively the entire embryogenesis [4] in the freshly laid egg — a hydroxylated form of β-carotene — is in fact "cis", which turns to "cis-trans" once the larva becomes vermiform. It also persists during the 5th embryonic stage. Esterification gets more marked during the red egg stage but sensibly diminishes as the larva reaches the 5th stage.

These modifications which take place in the molecular carotenoid constitution during embryogenesis point to the continuous molecular motion of one of the partners of the lipoproteic-carotenoid complex. At the same time, the lipids in this combination are also reactive, as we have already pointed out when measuring total lipids and fatty acids.

It should be recalled that although the analyses of the intact egg as well as of the caroteno-lipid complex pertain to the same stuff, the results

obtained differ in the two extracts in respect to the utilization of esterified fatty acids, respectively of the total ones. These differing results raise the question whether the utilization of esterified fatty acids in the caroteno-lipid complex is at odds with the remaining lipids in the egg.

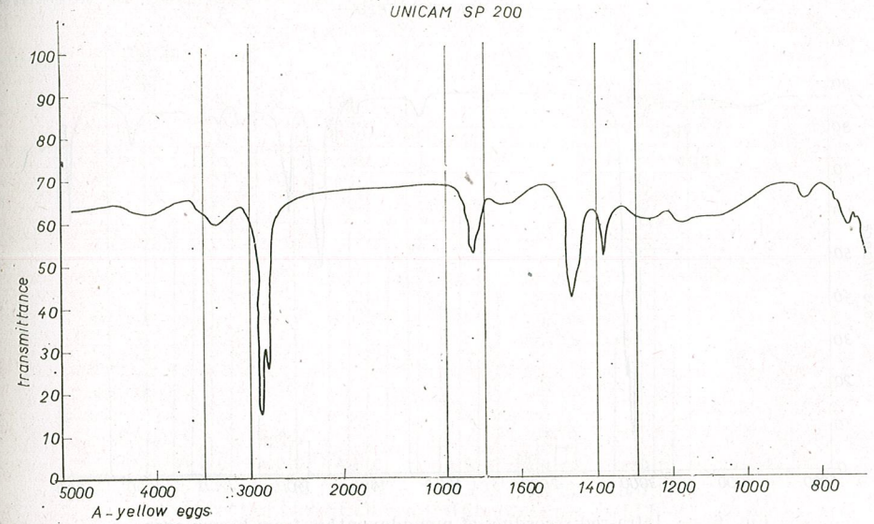

Fig. 4. — Infra-red recording of monadoxanthin from yellow eggs.

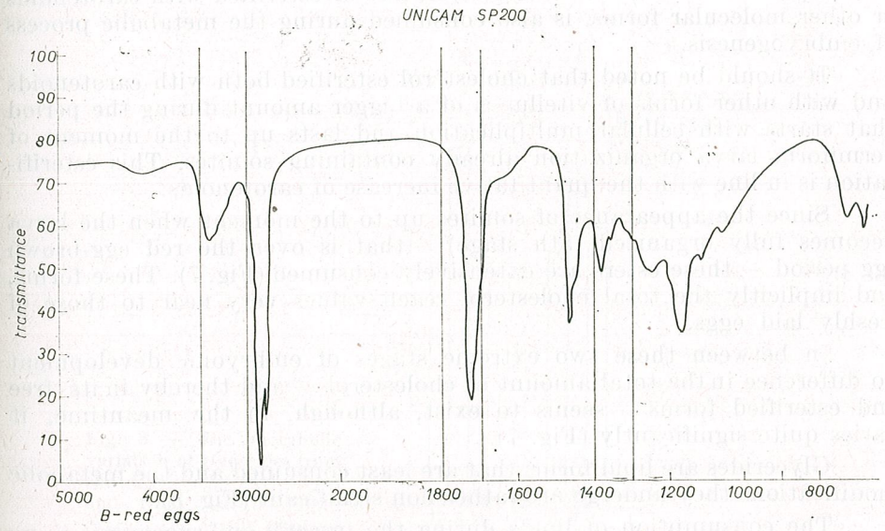

Fig. 5. — Infra-red recording of monadoxanthin from red eggs.

It may be said that the new larval tissues, according as they emerge, need the presence of special lipids with certain types of fatty acids. In this case the results obtained on analysing the full extract would represent the average of those two kinds of utilization.

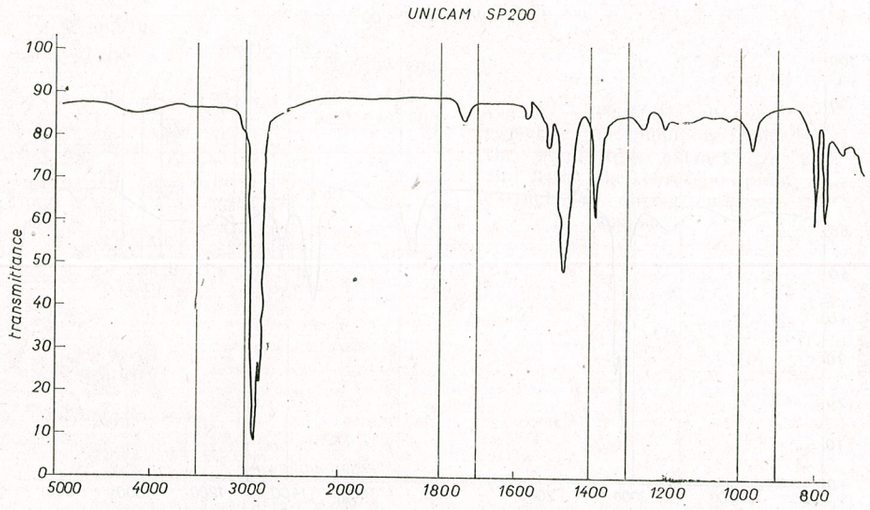

Fig. 6. — Infra-red recording of monadoxanthin from brown eggs.

Apart from this, total cholesterol, free or esterified with carotenoids or other molecular forms, is also consumed during the metabolic process of embryogenesis.

It should be noted that cholesterol esterified both with carotenoids and with other forms of vitellus is of a bigger amount during the period that starts with cellular multiplication and lasts up to the moment of vermiform larva organization already containing somites. This esterification is in line with the quantitative increase of carotenoids.

Since the appearance of somites up to the moment when the larva becomes fully organized (5th stage) — that is over the red egg-brown egg period — these esters are extensively consumed (Fig. 7). These forms, and implicitly the total cholesterol reach values very near to those of freshly laid eggs.

In between these two extreme stages of embryonic development no difference in the total amount of cholesterol — and thereby in its free and esterified forms — seems to exist, although, in the meantime, it varies quite significantly (Fig. 7).

Glycerides are lipid forms that are least consumed and the metabolic modifications they undergo are rather non significant (Fig. 8).

The consumption of lipids during the insect's embryogenesis is no ground for saying that it represents a primary energetic source or that

it is only a corollary to glucides probably consumed as the chief energetic material.

The actual decrease of carotenoid combinations and particularly of esterified forms makes us suppose that there is an evident and active participation of the caroteno-lipid complex — which constitutes the caroteno-lipo-proteic group of lipoproteins in the egg vitellus — to the formation of Colorado beetle embryo.

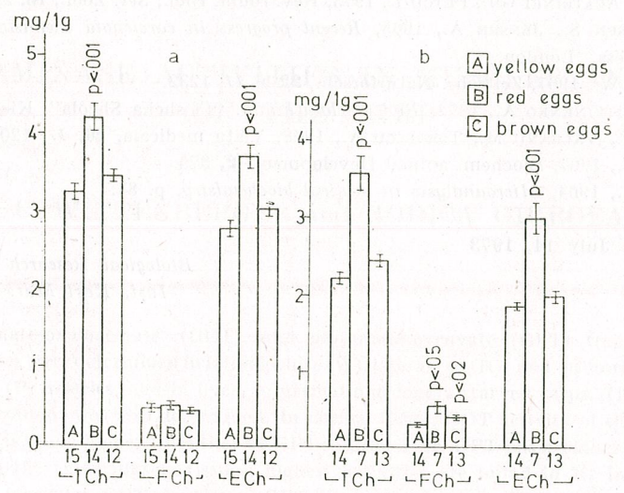

Fig. 7. — The metabolic variation of total cholesterol (TCh), free cholesterol (FCh) and esterified cholesterol (ECh) from the whole eggs extract (*a*) and from caroteno-lipid extract (*b*).

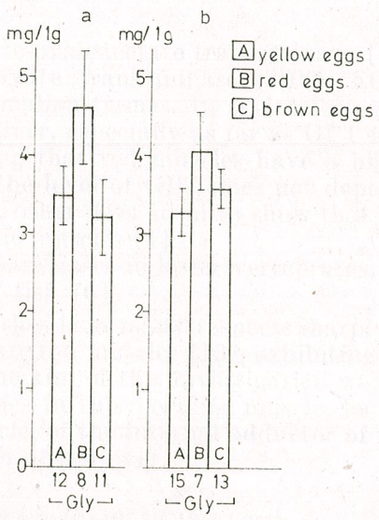

Fig. 8. — The metabolic variation of glycerides from the whole eggs extract (*a*) and from caroteno-lipid extract (*b*) during embryogenesis.

REFERENCES

1. Barreto R., Mano D., 1961, Clin. chim. Acta, **6**, *6*, 887.
2. Bonaly R., 1967, *Biosynthèse des caroténoïdes cycliques des levures du genre Rhodotorula.* Thesis, Nancy.
3. Cheesman D., Lee W., Zagalsky P., 1967, Biol. Rev., **42**, 132.
4. Jitariu M., Acatrinei Gh., Petcu I., 1973, Rev. roum. Biol., Sér. Zool., **18**, *2*, 145.
5. Liaaen-Jensen S., Jensen A., 1965, *Recent progress in carotenoid chemistry.* Pergamon Press, London.
6. Reichmuth W., 1967, Zeitschr. Naturforsch., **22 b**, *11*, 1232.
7. Sopin E., Litvinenko A., 1972, *Biologhichna himia.* " Vishcha Shcola", Kiev, 364.
8. Torgescu V., Văleanu M., Torgescu A., 1968, Viaţa medicală, **20**, *17*, 1201.
9. Williams J., 1967, Biochem. animal Development, **2**, 375.
10. Wootton A., 1964, *Microanalysis in medical biochemistry*, p. 84.

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TRANSAMINASE ACTIVITIES IN MUSCLES AND LIVER OF THE CARP

BY

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Glutamate-oxaloacetate (GOT) and glutamate-pyruvate (GPT) transaminase activities were determined in lateral white (W), lateral red (R), and pectoral fin adductor (P) muscles, and in liver, in normal and longly starved carps. Tissue glycogen content was also determined. In normal fishes, GOT activity of the muscle tissues is much higher than that of GPT; in the liver, GPT has a higher activity. Among the three muscle tissues, highest activities are found in R. In starved fishes, muscular GOT decreases, GPT increases; hepatic GOT increases, GPT decreases. Glycogen content drops during starvation only in R and L. The hypothesis of a muscular gluconeogenesis in R, via GPT, during starvation, is put forward.

The activities of glutamate-oxaloacetate transaminase (GOT, EC 2.6.1.1.) and of glutamate-pyruvate transaminase (GPT, EC 2.6.1.2.) are well studied in various mammalian tissues. In skeletal muscle activities are lower than in heart or in liver, especially as far as GPT is concerned [10]. There are data showing that red muscles have a higher GOT activity than white ones, while the level of GPT does not depend on the type of muscle [2] [3]. However, other data seem to show that both activities are somewhat higher in tonic muscles [4].

There are few data on transaminases in lower vertebrates, and especially few in muscular tissues of fish [6].

The lateral red muscle of fishes is in many respects sharply different from the general type of vertebrate red muscles [13], exhibiting metabolic similarities with the liver [1]. The aim of this investigation was to determine the GOT and GPT activities in this peculiar muscle, as compared to those of the lateral white muscle, of the internal adductor of the pectoral fin (ordinary red muscle), and of the liver.

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MATERIAL AND METHODS

Experiments were made on hatchery carps, weighing 250—350 g. The fishes were maintained in tanks with running water, at 10—14°C, without food. Two series of experiments were performed: on "normal" fishes, which have spent one month in the tanks, and on starved ones which have been maintained under these conditions during 12 months. All the experiments were made in November.

Immediately after decapitation of the fish, samples were taken from the lateral white muscle (W) of the epaxon part of the caudal peduncle, from the lateral red muscle (R) of the caudal peduncle, from the internal adductor muscle of the pectoral fin (P), and from the lobe of the liver (L) near the gall bladder. The samples were homogenized in a mortar with 10 cm^3. of saline for W, R, and P, and with 20 cm^3. for L. The following saline was used: KCl 180 mM, $MgCl_2$ 5 mM, potassium phosphate buffer 10mM; pH was 7.4. Enzyme activities were determined on the supernatant fluid of the tissue homogenate, after sedimentation without centrifugation [9]. The glycogen content of the same tissues (except P) was also determined [7].

RESULTS

Our results are given in tables 1 (GOT), 2 (GPT), and 3 (glycogen). Enzyme activities are expressed in micrograms of pyruvic acid per milligram of wet tissue weight during an incubation of 60 min. for the GOT, of 30 min. for the GPT, at 37°C.

Table 1

GOT activity of carp tissue homogenates, in micrograms pyruvic acid produced per milligram of wet tissue weight per hour. Values are given as means ± standard errors; in parentheses, number of individuals. W, white muscle; R, lateral red muscle; P, internal adductor muscle of the pectoral fin; L, liver; N, normal, S, starved fishes; D %, percentage difference between N and S; p, probability

	W	R	P	L
N	494±32 (10)	3000±168 (10)	1989±133 (10)	1067±85 (10)
S	84±5 (9)	108±12 (9)	134±24 (9)	5753±268 (7)
D %	−83	−96	−93	+439
p	<0.001	<0.001	<0.001	<0.001

Table 2

GPT activity of carp tissue homogenates, in micrograms pyruvic acid produced per milligram of wet tissue weight per 30 min. All other details as in table 1

	W	R	P	L
N	61±6 (12)	107±8 (12)	72±3 (12)	1521±141 (12)
S	213±19 (10)	2083±204 (9)	534±6 (6)	452±41 (10)
D %	+249	+1847	+642	−70
p	<0.001	<0.001	<0.001	<0.001

As one can see from tables 1 and 2, the activities of the investigated enzymes are highly different in various tissues. In normal fishes, the activities decrease in the following order:

for GOT: R > P > L > W;

for GPT: L > R > P > W.

In all muscle tissues, the GOT activity is much higher than that of GPT, while in the liver an inverse situation is observed.

In long-starved fishes, the activity ratios are drastically modified. In muscles, GPT activity is now much higher than GOT one; conversely, in liver the former drops below the value of the latter.

The glycogen content strongly decreases during the starvation, only in R and L (Table 3).

Table 3

Glycogen content of carp tissues, in micrograms per milligram of wet tissue weight. All symbols as in table 1

	W	R	L
N	2.80±0.19(7)	16.3±1.7 (7)	161.0±11.7 (7)
S	2.44±0.40(10)	10.1±0.7 (8)	61.6±5.4 (9)
D %	−13	−38	−62
p	>0.05	<0.01	<0.001

DISCUSSIONS

Our results indicate a distribution of the transaminase activities which is much different from that known in higher vertebrates. In mammalian tissues the GOT activity is higher than, or at least equal to, that of GPT, and both are higher in liver than in skeletal muscle tissue. The ratio of enzyme activities in white and red muscles, known in mammals and birds, is found also in fishes, as far as the GOT is concerned. This is true if we compare W both to P and to R. The P is known as a muscle in the structure of which red fibres make up the majority. It has typically tonic properties [5]. The R possesses peculiar structural and metabolic characteristics. It is to be stressed that clear-cut differences appear between the two red muscles (P and R) concerning transaminases: in normal fishes, both activities tested are 1.5 times higher in R than in P.

We have shown an inversion of the ratio of the two enzymes during starvation; this is true for both muscular and liver tissues. As far as we know, such a reversal has not yet been put into evidence. In the pectoral muscle of the pigeon a light decrease of the GPT activity during starvation has been described, but without any change in GOT [2]. Conversely, in the liver of fasted rats both alanine and glutamine concentrations decrease [8]; this shows an intensification of GPT activity. The same conclusion may be drawn when GPT is inhibited: the alanine concentration of the liver increases much more in starved than in normal rats, hence the GPT activity has been initially higher in the former case [11]. During starvation,

an imperious need for enhanced gluconeogenesis is felt by the organism, and the liver has the task to meet this need. It results that, in mammals, the transaminase participating in hepatic gluconeogenesis during starvation is the GPT.

The picture we obtained in the carp is different. Several hypotheses may be put forward to explain this difference. If we assume that among the carp tissues studied the liver is the unique site of gluconeogenesis during starvation, we must accept that the transaminase involved is GOT; indeed, the activity of this enzyme increases in the liver more than 5 times during starvation. On the contrary, if we assume that the gluconeogenetic transaminase is, in fish as in higher vertebrates, the GPT, we should admit that the enhancing of this process in fasted carps takes place not in the liver, but in the muscles. In this respect, the nearly 20-fold increase of GPT activity in R should be stressed. Of course, there is no strong evidence that enhancing of gluconeogenesis occurs at all in starving fish.

It is interesting to note that the long lasting starvation did not modify significantly the glycogen content of the W. This represents about 15% of the total glycogen of the fish body, in 1 — 2-year-old carps. On the contrary, glycogen content of L (about 80%) and of R(4—5%) are strongly affected. It seems that the fasting organism sacrifices the carbohydrate fuel reserve of R and L, maintaining at a constant level, as long as it is possible, that of W. This might support the view sustained by one of us, concerning the mainly non-locomotory function of the lateral red muscle in fish [12]. It is also to be observed that the modifications of transaminase activities do not parallel the changes of glycogen content of the respective tissue.

REFERENCES

1. Braekkan O.R., 1959, Rep. Technol. Res. Norw. Fish Ind., **3**, *8*, 1—42.
2. Braunstein A.E., 1949, *Biohimiia aminokislotnogo obmena*, Izd. Akad. Med. Nauk SSSR, Moscow.
3. Dawson D.M., Romanul F., 1964, Arch. Neurol., **11**, 369.
4. Kleine T., 1967, Z. klin. Chem. klin. Biochem., **5**, 244.
5. Litver G.M., 1956, Arhiv Anat. Ghistol. Embriol., **33**, 10—17.
6. Meşter R., Iordăchescu D., 1972, C.R. Acad. Sci. Paris, **275**, *10*, 1083—1086.
7. Montgomery R., 1957, Arch. Biochem. Biophys., **67**, 378—386.
8. Paleologos G., Muntwyler E., Kesner L., 1969, Proc. Soc. exp. Biol. Med., **132**, 270—271.
9. Reitman S., Frankel S., 1956, Amer. J. clin. Pathol., **28**, 56.
10. Schmidt E., Schmidt F.W., 1966, *Enzym-Fibel. Praktische Enzym-Diagnostik*. C.F. Boehringer u. Söhne GmbH, Mannheim.
11. Williamson D.H., Lopes-Vieira O., Walker B., 1967, Biochem. J., **104**, 497—502.
12. Wittenberger C., 1971, *Evoluţia funcţiei musculare la vertebrate*, Ed. Academiei, Bucharest.
13. — 1972, St. Cerc. Biol. Ser. Zool., **24**, *1*, 69—77.

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MODIFICATIONS OF THE THYROID AFTER HEMISPHERECTOMY IN PIGEONS

BY

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Hemispherectomy in pigeon produces ponderal modifications in thyroid. After hemispherectomy there develops a goiter 13 times heavier than the normal gland. The goiter is parenchymatous, non-homogeneous, hyperplastic, with numerous degenerative cellular lesions which are maximal a month after the operation. At the same time with the development of the goiter, the number of thyrotropic cells in the hypophysis increases and their activity intensifies.

The effects of hemispherectomy were investigated in birds by Ten-Cate [10] and Baiandurov [1].

Their investigations concern metabolic alterations, which are correlated with some neuroendocrine disturbances.

In our former researches there was found a delay in the development of the gonads and in the beginning of the sexual behaviour in males, as well as in the egg-laying in females [1].

Supposing that metabolic modifications and those connected with the reproductive function appear as a consequence of some endocrine troubles, determined by the absence of brain hemispheres, we proposed to investigate the structural modifications of the thyroid and hypophysis after hemispherectomy.

MATERIAL AND METHODS

The experiments were carried out on females of *Columba livia*, hemispherectomized at the age of 3 months. We extirpated both cerebral hemispheres, as shown in figure 1. After a week, two weeks, a month and a year we took out the glands and investigated them in comparison to

controls from animals of the same age and kept under identical conditions. The freshly removed glands were fixed in Bouin's fixative and then 6 μ slices were stained in trichrome azan. After hemispherectomy the thyroids underwent an accentuated hypertrophy, which determined us to estimate their weight.

RESULTS

In figure 2 the form and size of a thyroid lobe from a control animal (*a*) are shown in comparison with thyroids of animals hemispherectomized since a week (*b*), a month (*c*) and a year (*d*). After operation there is a progressive hypertrophy of the gland.

In the following we present the histological aspect of the thyroid in control and hemispherectomized animals.

In controls the thyroid lobe weighs 17.5 mg. The follicles (Fig. 3) have different sizes, their diameter ranging between 27 and 80 μ. Most of the follicles have a diameter of about 80 μ. The height of the epithelium reaches 5.55 μ. The lumen is full of colloid, with a border slightly crenellated by the resorption vacuoles. Most of the follicles have old and fresh colloid.

The general aspect of the gland suggests a moderate activity.

In pigeons hemispherectomized since a week, the thyroid lobe weighs 17.6 g. The gland has a non-homogeneous aspect (Fig. 4), as in the middle it contains microfollicles with a diameter between 16 and 18 μ, while at the periphery of the lobe persist large follicles. The height of the follicular epithelium increased to 9 μ, that is almost twice the control. The colloid decreased, having numerous resorption vacuoles. There is a hyperaemia of the blood vessels.

The histological images show that a week after hemispherectomy the thyroid has become more active.

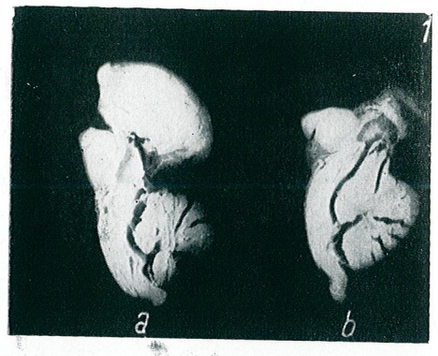
After two weeks strong structural modifications appear in the thyroid gland (Fig. 5), the aspect being more homogeneous due to the more uniform follicles. The follicular epithelium reaches 15 μ, nearly 3 times the control. Numerous nuclei are in mitosis (Fig. 6).

The basal cytoplasm of the epithelium cells suffers a strong vacuolization, which in some follicles leads to a separation of the epithelium from its basal membrane or even to its desquamation. The follicles are deprived of colloid. The hyperaemia of the blood vessels persists.

Two weeks after hemispherectomy there develops a parenchymatous, hyperplastic goiter without colloid, with hyperaemia, in which degenerative cellular lesions begin to appear.

A month after hemispherectomy, the thyroid lobe reaches the weight of 78 mg., i.e. four times the control. The histological aspect is profoundly modified as compared to the preceding lots. The thyroid gland takes again a non-homogeneous aspect (Fig. 7). At its periphery persists a reduced number of normal microfollicles without colloid, but the major part (about 80—90 %) of the gland is made up of microfollicles with an altered structure. Their epithelium is desquamated, so that the follicular struc-

PLATE I



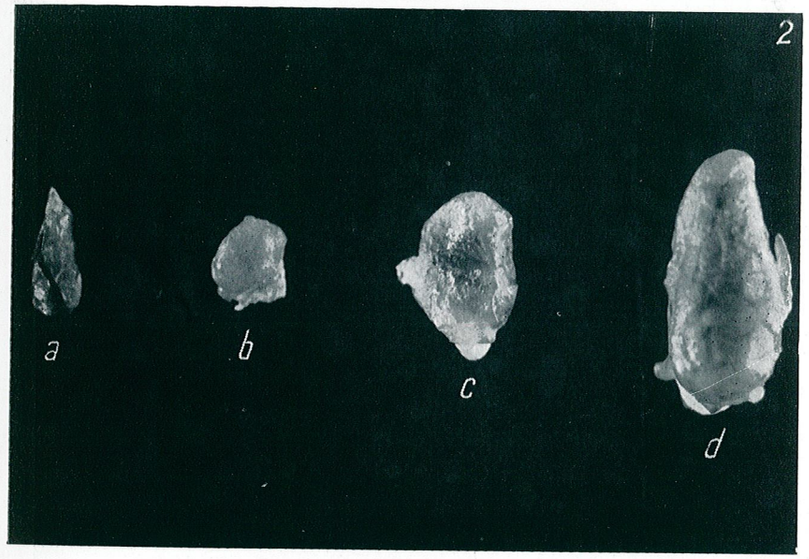
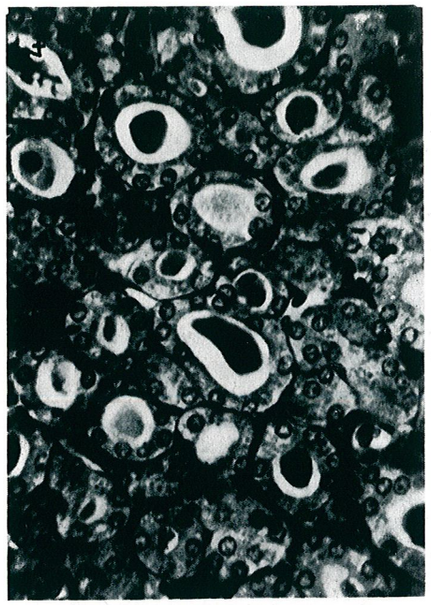


Fig. 1. — The brain of a normal (*a*) and a hemispherectomized pigeon (*b*), sectioned at the interhemispheric level.

Fig. 2. — The thyroid lobe of a control animal (*a*), an animal hemispherectomized since a week (*b*), a month (*c*) and a year (*d*).



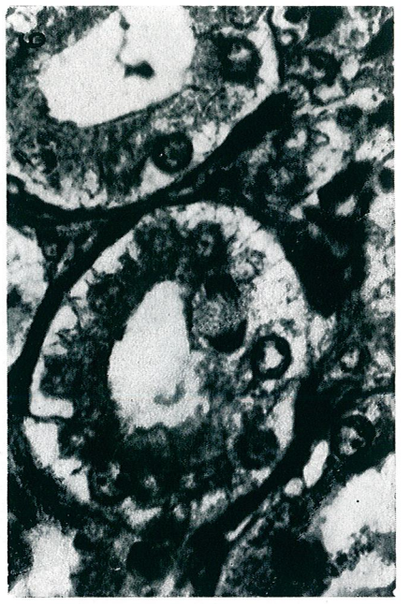
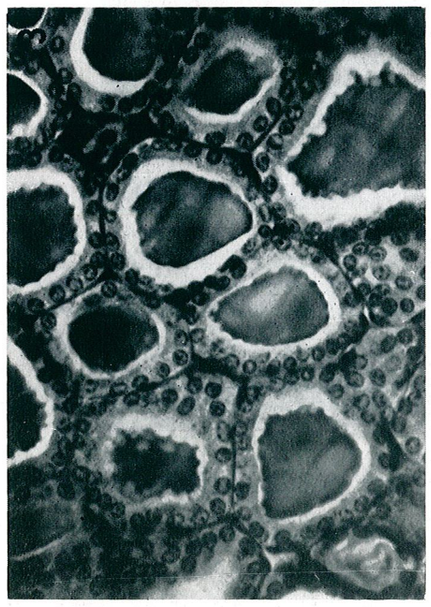


Fig. 3. — The thyroid of control animals. Col. azan, ob. × 40

Fig. 4. — The thyroid of an animal hemispherectomized since a week. Col. azan, ob. × 40.

Fig. 5. — The thyroid of an animal hemispherectomized since two weeks. Col. azan, ob. × 40.

Fig. 6. — The mitosis in the follicular epithelium of an animal hemispherectomized since two weeks. Col. azan, ob. × 90.



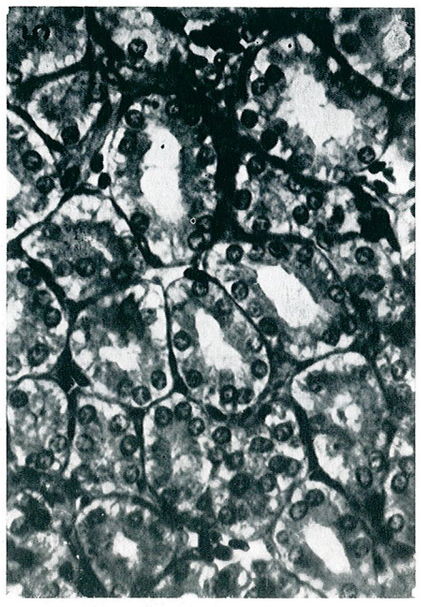


Fig. 7. — The thyroid of an animal hemispherectomized since a month. Col. azan, ob. × 40.

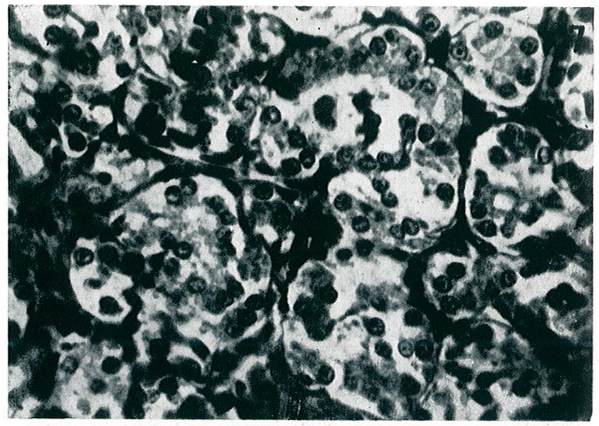


Fig. 8. — Hypertrophied thyroid follicles of an animal hemispherectomized since a year. Col. azan, ob. ×40.

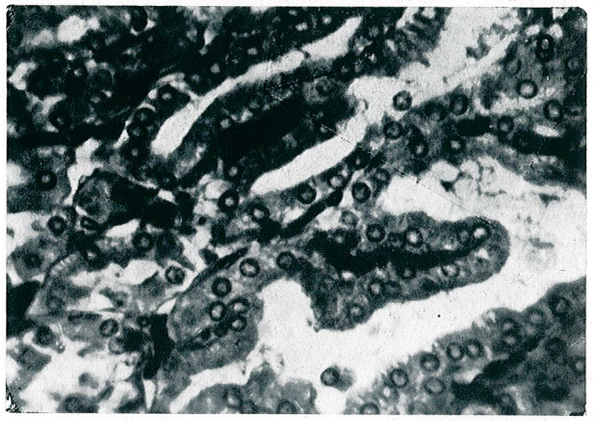
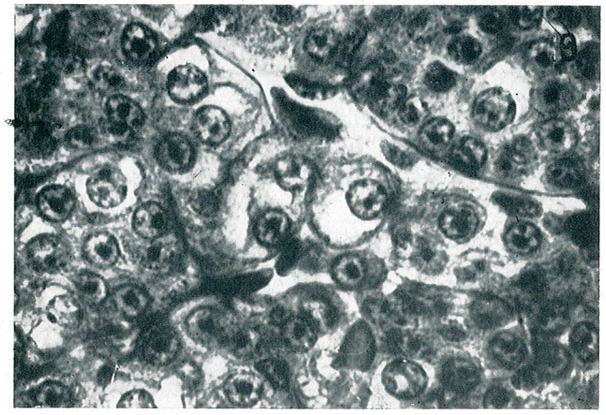


Fig. 9. — Delta basophil cells of the hypophysis of an animal hemispherectomized since two weeks. Col. azan, ob. × 90.



ture of the gland is hardly recognizable, rather by the conjunctive interfollicular septa and the dilated capillaries. Some nuclei of the desquamated epithelium become pycnotic. The colloid is absent. Thus, a month after hemispherectomy the hypertrophy of the gland increases. The histological picture shows a non-homogeneous goiter, formed of many microfollicles with a desquamated epithelium and numerous degenerative cellular lesions, as well as of some normal macrofollicles at the periphery of the gland, without colloid and with hyperaemia of the blood vessels.

A year after hemispherectomy, the thyroid lobe reaches a weight of 230 mg., 13 times that of the control (Fig. 2 *d*). The non-homogeneous aspect of the gland persists and is even accentuated.

The interior of the gland is formed of microfollicles, some of them normal, but the great majority presenting degenerative cellular lesions due to the detachment of the epithelium from its basal membrane or to the desquamation of the epithelium which falls in the follicular lumen, the same as in animals hemispherectomized since a month. Interesting is the periphery of the gland with unusually large, hypertrophied follicles (Fig. 8). The secretory and absorptive surface of the follicular epithelium has increased not only by the hypertrophy of the follicles, but also by its folding. The height of the hypertrophic follicles is 13 μ. The colloid is missing or is in a very small quantity, being strongly resorbed on the borders. The vasodilatation is so intense, that there are extravasations, the blood invading even some thyroid follicles. The histological pictures show that a year after hemispherectomy there occurs a parenchymatous, hyperplastic, non-homogeneous goiter with degenerative cellular lesions in the interior of the gland and with hypertrophic follicles at the periphery, without colloid, accompanied by vasodilatations and extravasations.

The thyroid activity is under the thyrotropic control of the hypophysis [8]. After Tixier-Vidal et al. [11], who studied systematically the cell types of the pigeon hypophysis, the delta basophil cells, stainable with aniline-blue, are implicated in the secretion of the thyrotropic hormone.

After hemispherectomy a series of modifications appear at the level of the delta cells in the hypophysis. There is an increase of their number and they become hypertrophic. The most remarkable manifestations appear two weeks after hemispherectomy, when the cells present the most evident signs of activation. They become hypertrophic, their large nucleus has a voluminous nucleolus, while the cytoplasm degranulates progressively and finally undergoes a strong vacuolization. The vacuoles, small at first, fuse and form 1—2 big vacuoles which push the nucleus and the cytoplasm to a side of the cell (Fig. 9). At the same time with the cytoplasm vacuolization, the nucleus shows signs of exhaustion, the nucleolus decreases and finally the normal structure of the nucleus is altered and we assist to the destruction of the cell by holocrine secretion. These aspects reveal an accentuated hypersecretion of the thyrotropic hormones.

A year after hemispherectomy the thyrotropic activity of the hypophysis persists. The hypertrophied delta cells remain in a great number, with a tendency to degranulation, but pronounced vacuolizations and manifestations of holocrine secretion are no longer occurring.

DISCUSSIONS AND CONCLUSIONS

Our researches show that in birds hemispherectomy has profound influences on the thyroid gland. After hemispherectomy a goiter appears which develops progressively, so that a year after the operation the weight of a thyroid lobe becomes 13 times greater than that of a control animal.

The histological examination shows that the goiter has a parenchymatous, non-homogeneous, hyperplastic character, without colloid and with numerous degenerative cellular lesions. The intensity of these alterations becomes maximal a month after hemispherectomy. The goiter is accompanied by vasodilatation, that after a year leads to extravasation and infiltrations of blood in the thyroid follicles.

The histological examination of the hypophysis explains the appearance and the excessive development of the goiter. After hemispherectomy the number of the thyrotropic cells increases and they become hypersecretory. The hypersecretion of the thyrotropic hormone seems to attain the maximum, even anarchic, intensity two weeks after hemispherectomy.

Our results are in concordance with those obtained by other authors [5], [8]. This shows that the function of the thyroid is at a great extent controlled by the hypothalamic-hypophyseal system, being dependent also on the integrity of other zones, situated beyond the hypothalamus. The extrapyramidal zone and the rhinencephalon intervene, as stated for rats [3], [5], in the control of the thyrotropic hormone secretion, their destruction leading to a strong discharging of the latter. Lupulescu et al. [3] suppose the existence in these zones of some centres which inhibit the TSH secretion of the hypophysis and whose destruction leads to a massive discharge of thyrotropic hormone.

By analogy, we suppose, since we extirpated these regions, that the modifications described by us in the thyroid gland have similar reasons.

Our results are contrary to the findings of Lizzi et al. (quoted by [1]) and to the suppositions of Baiandurov [1] who considers that the pituitary is not involved in these processes.

REFERENCES

1. Baiandurov B.I., 1949, *Trofichescaia functsia golovnogo mozga*, Moscow.
2. Hurduc M.I., 1966, St. Cerc. Embriol. Citol. Ser. Citol., **3**, *1*, 3.
3. Lupulescu A., Nicolescu-Catargi Al., Merculiev E., Petrescu S., 1963, St. Cerc. Endocrinol., **14**, *3*, 379.
4. Milcu St., Belloiu D., Holban R., Săhleanu V., 1956, St. Cerc. Endocrinol., **7**, *3*, 481.

5. Milcu St., Lupulescu A., Săhleanu V., Holban R., 1963, *Fiziopatologia experimentală a glandei tiroide*. Ed. Academiei, Bucharest.
6. Parhon C.I., Pitiș M., Stănescu V., Ionescu V., 1963, St. Cerc. Endocrinol., A, 131.
7. Pora E.A., Persecă T., Lungu Al., 1966, St. Cerc. Biol. Ser. Zool., **18**, *6*, 491.
8. Săhleanu V., Holban R., Belloiu D., 1957, St. Cerc. Endocrinol., **8**, *2*, 213.
9. — 1958, St. Cerc. Endocrinol., **9**, *4*, 480.
10. Ten-Cate J., 1936, Ergebnisse der Biologie, **13**, 93.
11. Tixier-Vidal A., Assenmacher I., 1966, Zellforsch. mikrosk. Anat., **69**, 498.

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THE INTERSPECIFIC HYBRIDS BETWEEN KURDISTAN HAMSTER *(MESOCRICETUS BRANDTI)* AND GOLDEN HAMSTER *(MESOCRICETUS AURATUS)*

BY

P. RAICU, MĂRIUCA NICOLAESCU and MARIA KIRILLOVA

From crossings between *Mesocricetus brandti* ♀ and *Mesocricetus auratus* ♂ hybrids were obtained which presented characters intermediate between the parents, having the chromosomal number (2n = 43). The karyotype presents two distinct chromosome sets corresponding to those in the two parent species. In the male meiosis we could identify only prophase I probably because of the absence of homology between the chromosomes of the two species. The sex-chromatin in male is rather frequent. The karyotype and the idiogram in *Mesocricetus brandti* were also performed.

The hamsters became lately first order laboratory animals because of some important characteristics : they can be easily bred and multiplied, they reach soon the maturity, they can be inbred, they give lines of a high homozygosis level, and they have chromosomes fit for the cytogenetic researches. The first species of hamsters introduced into the laboratory was the golden hamster *(Mesocricetus auratus)*, after which followed the Chinese hamster *(Cricetulus griseus)*, the Romanian hamster (*M. newtoni*) and recently, the Kurdistan hamster *(M. brandti)*.

Recently there were also obtained some interspecific hybrids between : *M. auratus* × *M. newtoni*, by Raicu and Bratosin [5], Raicu, Ionescu-Varo and Duma [6], Todd et al [9]; *M. newtoni* × *M. brandti*, by Raicu et al. [8]; *M. newtoni* × *M. brandti*, by Todd et al. [9].

Taking into account that the hybrid animals present a great importance for the immunologic studies and for those referring to the homology of the chromosomes and the relationship level between the various species, we necessarily considered to continue our researches begun in 1968 concerned with the hybrids production and with their cytogenetic investigation.

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MATERIAL AND METHODS

Animals originating from the colony of the Genetics Department, University of Bucharest, belonging to the species: golden hamster *(Mesocricetus auratus)* 2n = 44, Kurdistan hamster *(M. brandti)* 2n = 42, and the interspecific hybrids from F_1 (2n = 43), were used.

We made comparative researches on the karyotypes of the two species and of the hybrid animals from F_1. We also performed the idiogram of the species *M. brandti* and we studied the meiosis in the hybrid animals *M. brandti* ♀ × *M. auratus* ♂. The reciprocal hybridization failed.

The method used was the following: the animals were injected i.p. with a 0.04 % colchicine solution two hours before killing. Bone marrow from both femurs was used. The obtained cellular suspension underwent hypotony treatment with 0.75 % citrate solution of Na, fixation in methanol acetic acid (3 : 1) and the preparations were carried out according to the usual air drying technique. The preparations were coloured with a Giemsa solution.

The meiosis was studied in the testes which were immersed in a hypotone citrate solution of Na, and were then fixed and coloured as we have described above.

The sex chromatin was studied in the hepatic cells, the material being fixed in methyl alcohol — acetic acid (3 :1) for 30—60 minutes. The fixed material was passed in two successive baths of alcohol 50 % and 70 % and then hydrolized in a 4 N HCl for 12 minutes at room temperature.

The preparations were washed in distilled water and then coloured several times in 1 % cresyl violet solution.

In order to carry out the idiogram of *M. brandti* we performed 10 karyotypes for each sex, we made measurement studies and we classified the chromosomes in different types in relation to the arm ratio (long/short) according to the nomenclature established by Levan, Fredga and Sandberg [3].

RESULTS AND DISCUSSION

The interspecific hybridization between *M. brandti* ♀ and *M. auratus* ♂ is very difficult and the number of offsprings is very small. Thus, for a total of 10 females mated and submitted to copulation, we have obtained only 4 hybrids, that is 0.4 offsprings for a female. Normally in the parental species the number of offsprings for a female is 7—8. The phenotype of the hybrids is intermediary between the parents.

For the cytogenetic study of the hybrids it was first necessary to investigate the chromosomal complement of the parents. While in the golden hamster the karyotype was well studied, for the Kurdistan hamster researches were carried out only by Lehman and Macpherson [2] and Todd et al. [9] and were not quite conclusive.

The biometrical studies of the chromosomal complement in *M.brandti* (2n = 42) showed that it is made of 20 pairs of autosomes with a length varying between 2.84 and 10.03μ, while the heterosomes are represented by a metacentric X chromosome of great size (9.02μ) and by a submetacentric Y chromosome of medium size (7.15μ). Among the 20 pairs of autosomes, only one (the pair 17) is made of metacentric chromosomes, the others being submetacentric. These researches accurately permitted to establish the types of chromosomes and respectively the idiogram. They demonstrated, among others, that the pair number 20 is represented by

submetacentric chromosomes with the arm ratio 2.44 and not by acrocentric ones, as stated by Lehman and Macpherson [2] and Todd et al. [9].

The meiosis study in the male of *M. brandti* showed that in the first metaphase and in the diakinesis the chromosomes form 21 bivalents of different types: ring, cross, rod. As regards the sex chromosomes, they present an end-to-end type of association similar to that produced in *M. auratus*, which was studied by Fredga and Santesson [1], and to that studied by Raicu, Nicolaescu and Kirillova [7] in *M. newtoni*.

Mesocricetus auratus species presents 2n = 44, the chromosomal complement being made of 21 pairs of autosomes out of which 17 pairs of meta-, submeta- and subtelocentrics and four pairs of metacentrics, while the heterosomes X and Y are submetacentric.

The male hybrids present an intermediate number of chromosomes (2n = 43) between the parental species, their karyotype permitting the accurate identification of the two chromosomal groups of different origin. As concerns the heterosomes, the X chromosome is a big metacentric inherited from the species *M. brandti* and the Y chromosome is a submetacentric of big size proceeded from the species *M. auratus*.

Because of the great size of X heterosome both in *M. auratus* and in *M. brandti*, Ohno [4] considered that they are formed by a duplication process so that they contain about 10 % of the whole amount of genetic material instead of 5 %. That is why in the females of the respective species one can notice the relatively frequent presence of the nuclei with 2 sex-chromatins different in size, which possibly have their origin in the heterochromatinization of a whole X chromosome and of half of its homologue [7].

The sex-chromatin study in the male hybrid of *M. brandti* ♀ × *M. auratus* ♂ showed a rather great frequency of the nuclei with one sex-chromatin (36.25 %) and with 2 sex-chromatins (8.22 %).

Table 1

The sex-chromatin frequency in the hybrid *M. brandti* ♀× *M. auratus* ♂ in liver

The number of the studied nuclei	The nuclei frequency			
	Without sex-chromatin	With one sex-chromatin	With 2 sex-chromatins	With 3 sex-chromatins
5961	1063	709	161	23
100 %	54.36	36.25	8.22	1.17

The sex-chromatins result from the heterochromatinization and the genetic inactivation of half of the X duplicated chromosome, which is also of a great size.

Our researches concerning the meiosis in the interspecific male hybrid permitted the identification only of prophase I and failed to recognize any diakinesis or metaphase I, or any other subsequent phases. The absence of a homology between the chromosomes of the two species ex-

plains the impossibility of the bivalents to be formed during meiosis and the fact that the reductional division is stopped before metaphase I. Consequently, the hybrids are sterile and all our attempts to use these males for the back cross with females from the two species did not succeed. The spermatogenesis in hybrids is likewise absent. These researches are in agreement with our previous studies [6] [8] carried out in the hybrids *M. newtoni* × *M. auratus* and *M. newtoni* × *M. brandti*, which demonstrated the sterility of the hybrids and its cytological and histological causes.

We can therefore conclude that the hybridization test demonstrates that the species used as genitors in the respective study are distinct, well-individualized species from the genetic point of view. The comparative study of the karyotypes of the two species confirms our supposition.

Table 2

The biometric study of karyotype in *Mesocricetus brandti* (2n=42)

Chromosome No.	Absolute length in μ			Relative length (nA+X)‰	Arm ratio L/S	Chromosome type
	Mean standard ♂	Error	Limits			
1	10.03 ±	0.20	7.63—13.46	67.20	2.13	SM
2	9.15 ±	0,28	6.92—11.92	61.31	2.13	SM
3	8.84 ±	0.19	6.64—11.15	59.23	2.15	SM
4	8.40 ±	0.16	6.54—10.38	56.28	2.17	SM
5	8.36 ±	0.22	6.54—9.80	56.01	2.27	SM
6	7.96 ±	0.16	5.96—9.61	53.33	2.33	SM
7	7.95 ±	0.26	6.16—10.00	53.28	2.39	SM
8	7.73 ±	0.16	5.38—9.61	51.79	2.24	SM
9	7.44 ±	0.15	5.76—9.61	49.85	2.10	SM
10	7.30 ±	0.15	6.16—10.00	48.91	2.38	SM
11	7.25 ±	0.14	5.57—8.84	48.57	2.25	SM
12	7.01 ±	0,13	5.38—8 84	46.97	2.16	SM
13	6.60 ±	0.13	5.38—8.04	44.22	2.20	SM
14	6.34 ±	0.37	5.19—7.50	42.48	2.18	SM
15	6.10 ±	0.13	4.61—7.69	40.87	2.19	SM
16	5.76 ±	0.13	4.23—6.92	38.59	2.15	SM
17	5.34 ±	0.03	3.26—6.15	35.78	1.67	M
18	5.30 ±	0.03	3.65—6.15	35.51	1.96	SM
19	4.52 ±	0.11	3.25—5.76	30.28	1.76	SM
20	2.84 ±	0.69	2.30—3.84	19.02	2.44	SM
X	9.02 ±	0.23	6.92—11.88	60.43	1.33	M
Y	7.15 ±	0.46	4,61—9.42	47.90	2.38	SM

Our previous studies in the hybrid *M. brandti* × *M. newtoni* showed the presence in metaphase I of meiosis of 16 bivalents and 2 tetravalents, which indicates a high level of homology between the chromosomal sets of the two species [8].

In this way we can conclude that *M. brandti* is more related to *M. newtoni* than *M. auratus*.

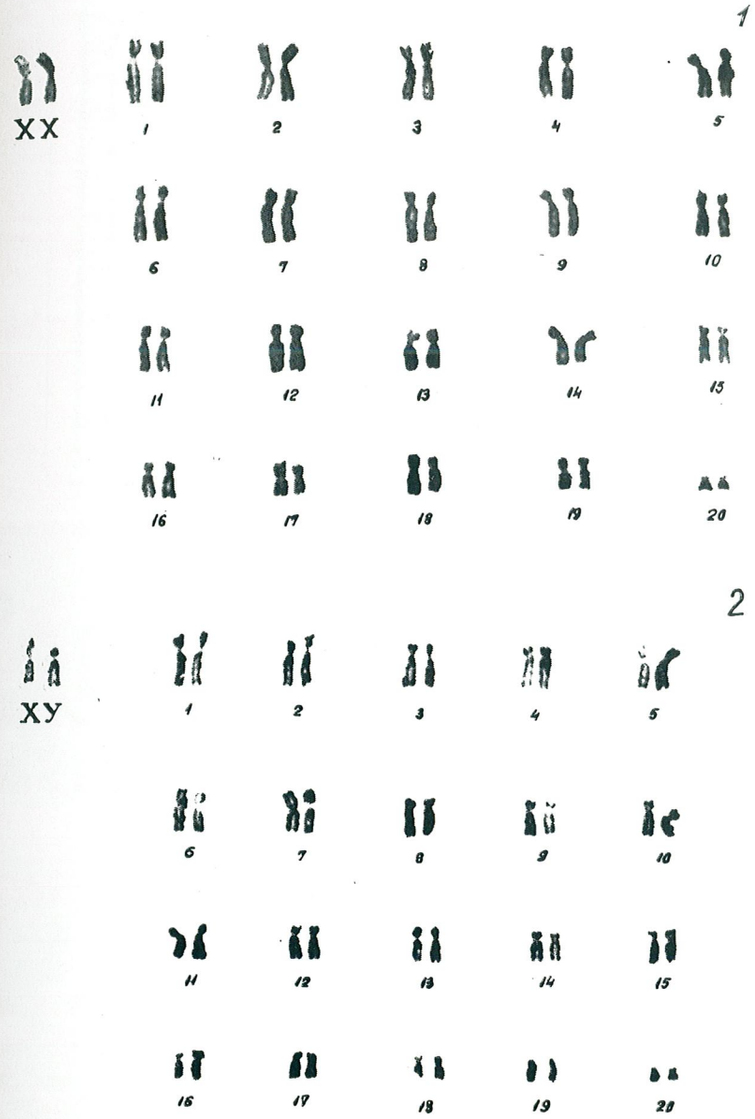

Fig. 1. — Female karyotype of *Mesocricetus brandti*.

Fig. 2. — Male karyotype of *M. brandti*.

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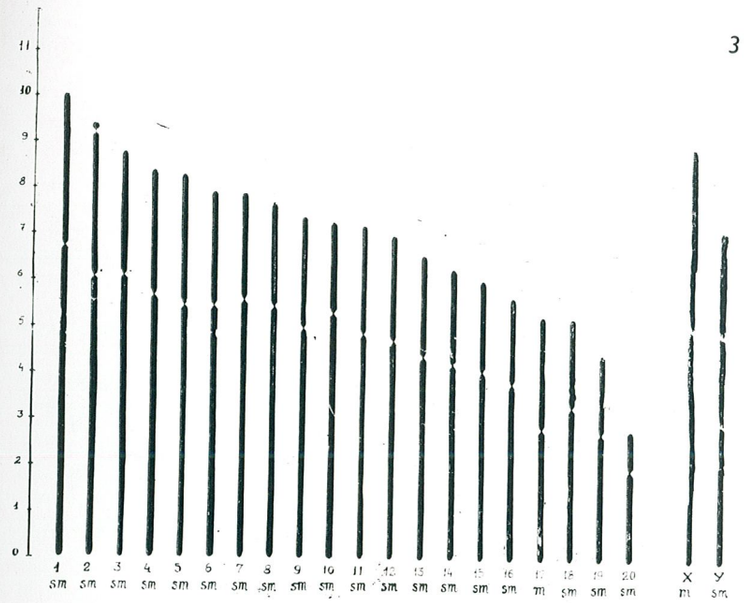

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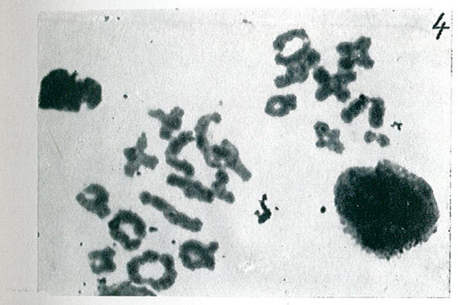

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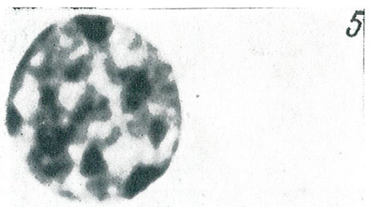

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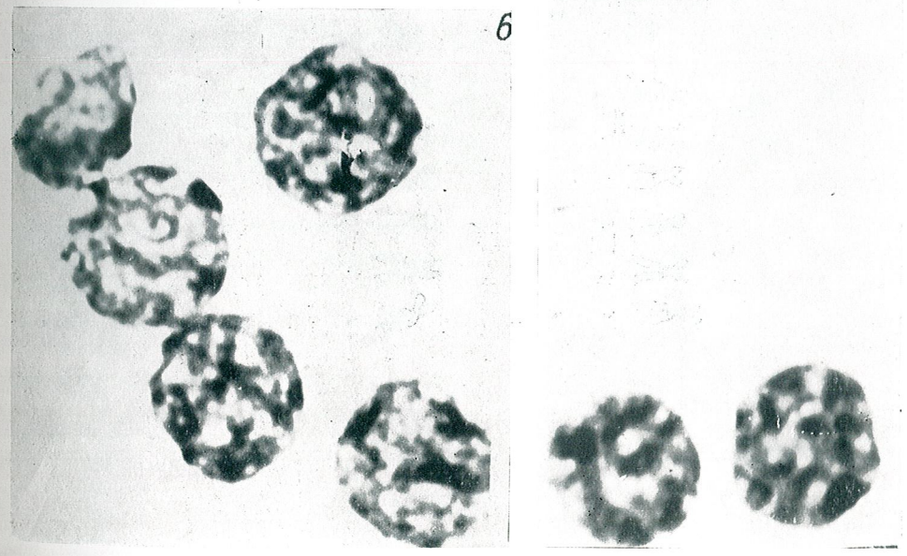


Fig. 3. — Idiogram of *M. brandti*.

Fig. 4. — Diakinesis of meiosis in *M. brandti*.

Fig. 5. — Nuclei with sex-chromatin in female of *M. brandti*.

Fig. 6. — Nuclei with sex-chromatin in male of *M. brandti*.

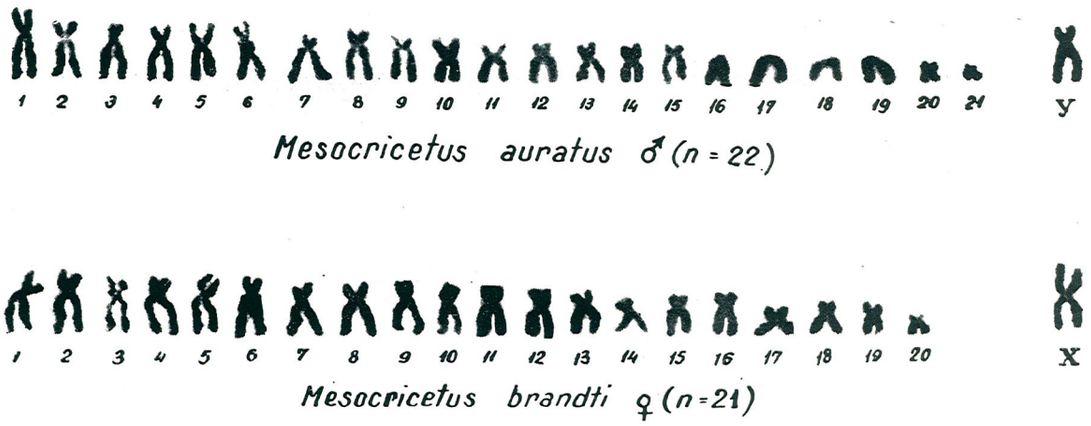
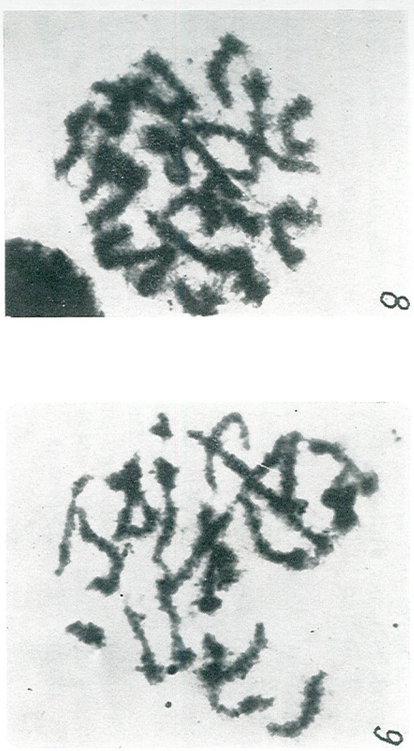


Fig. 7. — Male karyotype in hybrid of *M. brandti* ♀ × *M. auratus* ♂.



Figs. 8 and 9.— Prophase I of meiosis in a male hybrid.

Fig. 10. — Nuclei with sex-chromatin in a hybrid male.

KARYOTYPE OF TRANSPLANTABLE TUMOURS INDUCED WITH 3,4-BENZPYRENE IN *MESOCRICETUS AURATUS*

BY

AGRIPINA LUNGEANU

Subcutaneous tumours were induced by injecting a unique dose of 0.03 μg 3,4-benzpyrene in newborn golden hamsters. The cytogenetic study of primary tumours and of 60 tumours transplanted *in vivo* for 33 generations reveals a very great karyotype heterogeneity. We consider that in the case of these chemically induced tumours, the karyotype evolution occurred both by morphological restructuring of the chromosomes and by polyploidization. The genetic regulation of cell division was unquestionably affected.

The cancerigenic action of chemical substances was accidentally observed as early as the 17th century in people who had malignant tumours due to the repeated contact with tar, during work of a peculiar kind. Then, the cancerigenic action of numerous chemical substances of quite different nature was experimentally proved on different laboratory animals. Among these, 3,4-benzpyrene was proved to be one of the powerful cancerigenic substances belonging to the category of polycyclic hydrocarbons. The cancerigenic action of 3,4-benzpyrene in the golden hamster was studied by Gye and Foulds [3]. Di Paolo and coworkers [2] have recently presented the histopathologic aspect, the heterotransplantability, growth capacity and karyotype of primary subcutaneous tumours induced by a single injection of 3,4-benzpyrene, in Syrian hamster.

The present paper records observations concerning chromosomes and the karyotype of tumours induced with 3,4-benzpyrene (BP) in the golden hamster, as well as their course during *in vivo* transplantation.

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MATERIAL AND METHODS

They were described in detail in a previous work [8].

RESULTS

The cytogenetic study of 4 primary tumours was carried out and the evolution of the tumours karyotype on a strain transplanted *in vivo* for 33 passages was followed up. Subcutaneous tumours were obtained in a 100 % proportion at the level of all passages. Out of 202 tumours in different evolution stages that were under study, good cytogenetic preparations were obtained only in 60, as it is difficult to grasp the optimum phase of cell division. In primary tumours, the cytogenetic study was performed with a view to establishing the incidence of cells with different ploidy degree. Transplantability was studied in order to follow up the evolution of the tumour karyotype, to detect the moment of its stabilizing.

Primary tumours were denoted by BP2, BP5, BP6 and BP7, where BP = benzpyrene and 2, 5, 6, 7 represent the number of the tumour in order of the sacrification. Out of induced primary tumours, only those four could be studied, though 90 % of animals inoculated with the cancerigenic substance evinced a positive response. Strains from primary tumours were preserved, were the detailed cytogenetic stupy and karyotype determination were performed, using them as term of comparison for transplanted ones.

BP2 tumour. Removed from a female bearer, it has 13 polyploid metaphases with more than 100 chromosomes, out of 48 studied metaphases. Hypertriploidy (67—70 chromosomes) prevails in this tumour.

BP5 tumour. It grew on a female animal. Out of 40 metaphases, polyploids represent 35 %, having 78—86 chromosomes. The presence of two stem-lines is observed, rather closely grouped round aneuploidy (60 % of the total of analysed metaphases) as well as hypotetraploidy. The anarchic multiplication of the chromosomes is also observed in this tumour.

BP6 tumour. The bearer is a female hamster. It shows 40 diploid metaphases of a total of 118 analysed ones. Polyploid cells ranging between 80 and 88 chromosomes represent 31 %. Aneuploidy is present in a very low percentage (9 %).

BP7 tumour. The tumour developed on a male hamster. It is the only primary tumour with a high proportion of diploid cells (apparently diploid) : 38 %. The incidence of polyploidy is very low as against the other primary tumours (Fig. 2).

Transplanted tumours. The present paper records the evolution of the karyotype in the strain originating from the BP5 tumour.

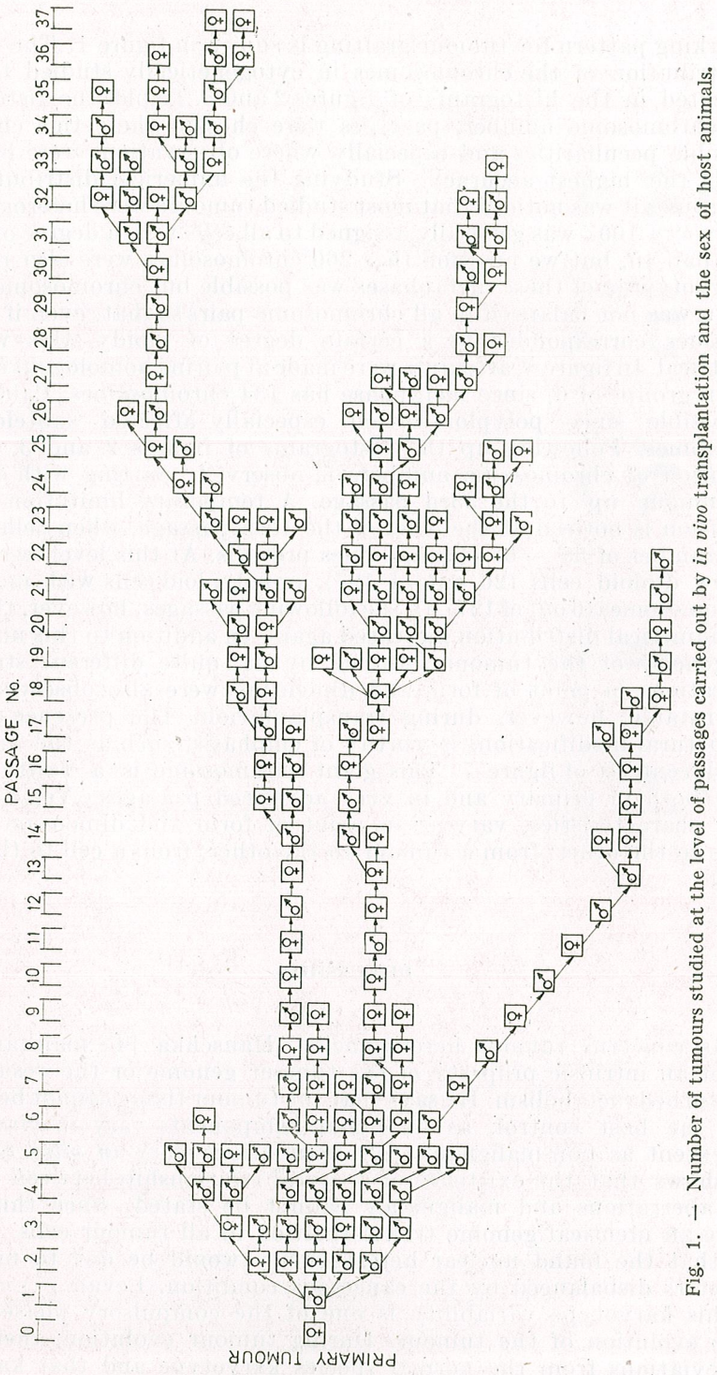

Fig. 1. — Number of tumours studied at the level of passages carried out by *in vivo* transplantation and the sex of host animals.

The working pattern for tumour grafting is shown in figure 1. The numerical distribution of the chromosomes in cytogenetically studied tumours is presented in the histograms of figures 2 and 3. To plot the distribution of the chromosome number, passages were chosen where this character had visible peculiarities and especially where observations were performed with the highest accuracy. Studying the numerical distributions of chromosomes it was noticed that most studied tumours were heterogeneous. The sign ">100" was generally assigned to all cells with a degree of ploidy higher than 4n, but we mention that 260 chromosomes were also reached. The karyotyping of these metaphases was possible but chromosome multiplication was not balanced in all chromosome pairs so that, even if numerical values corresponded to a certain degree of ploidy, this was not symmetrical. In figure 5, attempts were made at pairing homologous chromosomes in groups of 6, since metaphase has 134 chromosomes. Pairing was not possible since polyploidization especially affected subtelocentric chromosomes. Following up the histograms of figures 2 and 3, a wide distribution of chromosome numbers is observed, starting with the primary tumour up to the 33rd passage. A temporary limitation of this distribution is noticed at the level of the 19th passage, when cells with a modal number of 58 — 66 chromosomes prevails. At this level were found very few diploid cells (20 out of 127), or polyploid cells with more than 100 chromosomes (6 out of 127). In the following passages, however, the range of the numerical distribution increased again. In addition to this numerical heterogeneity of the tumours studied by us, quite different structural modifications in point of form and dimension were also observed. They were unstable, however, during transplantation. The presence of striking structural modifications is worthy of emphasis, such as the dicentrics and polycentrics of figure 7. This giant chromosome is a restructuring, present both in primary and in very advanced passages. Yet it has no marker characteristics, varying in point of form and dimension from a passage to the other, from a tumour to the other, from a cell to the other (Fig. 7).

DISCUSSIONS

As concerns tumour heterogeneity, Hauschka [4] maintains that it is not an intrinsic property of the tumour genome or the resultant of the disturbed metabolism. He says that the tumour tissue cannot be inhibited by the host control, as its clonal components vary *in vivo* to the same extent as non-malignant cell populations vary *in vitro*. Sandberg [11], shows that the existence of a causal relationship between chromosomal aberrations and malignancy cannot be stated, since this would suppose an identical genome transformation in all tumour cells. He supposes that the found nuclear heterogeneity would be due to metabolic parameters disbalanced by the cancerous condition. Levan [7] considers that this karyotype variability is one of the compulsory phases of the genetic evolution of the tumour. During tumour evolution, there occur first deviations from the normal species karyotype and that karyotype

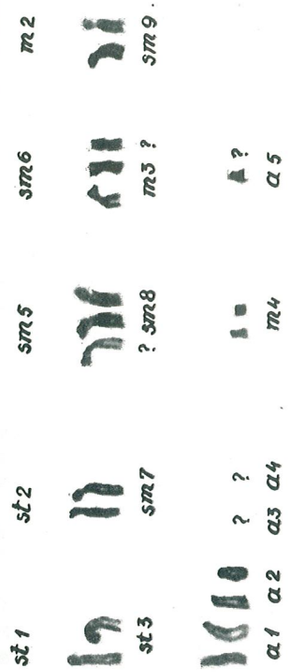

Fig. 4. — Karyotype and metaphase of hypodiploid cell in the BP5 primary tumour. Chromosomal restructurings are present, denoted by Di in the karyotype.

Fig. 5. — Karyotype of a hyperhexaploid cell (6n=132) with 134 chromosomes + 8 minute chromosomes (mi) from the tumour of the 9th passage.

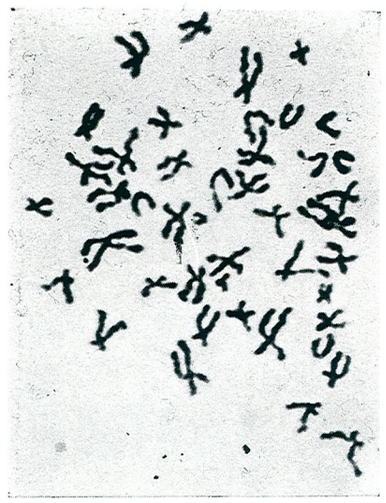

Fig. 6. — Karyotype and metaphase of a pseudo-diploid cell from the tumour of the 33rd passage.

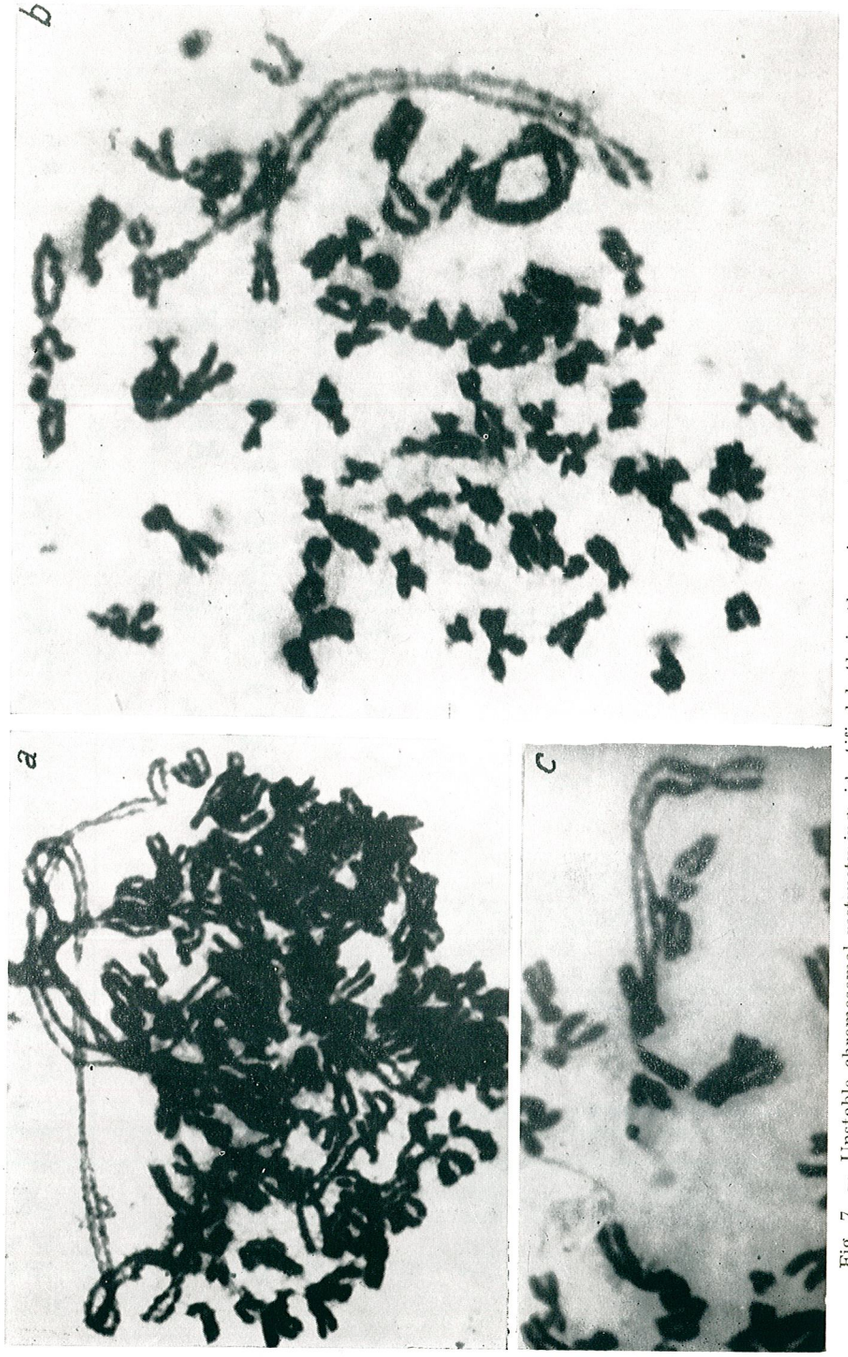

Fig. 7. — Unstable chromosomal restructurings identified both in the primary tumour and at other development levels.

diversity is gradually set on. It is emphasized as the tumour advances and it serves as a material for evolution by clonal selection. Ohno [9] points to the genetic implication of malignant somatic cells instability. Malignant cells are not alien to the organism, deriving from normal cells. As against somatic cells with a limited life, tumour cells acquire an exponential growth capacity, becoming organisms by themselves and being submitted to laws that govern the organism evolution. Genetically speaking, malignant cells are aneuploid. Whether aneuploidy is a cause or a consequence of malignancy, is still to be found out. Rei Kato and Levan [10] state that the chromosomal imbalance, both numerical and structural, is one of the first signs of malignancy. Weinstein [12] admits the possibility that all tumours in their initial development stage have differentiation aberrations that do not imply permanent changes of the cell DNA. He asserts that a very important part in chemical cancerization is played by RNA, where linking would be preferential.

As concerns the mechanism of maintaining structural modifications produced by cancerization with 3,4-benzpyrene, Lesko [5], [6] experimentally proves the preferential covalent linking of benzpyrene in the 8th position of guanosine from DNA and RNA. He supposes that the preferential linking of cancerigenic substances to RNA would despiralize its conformation, altering the interaction with amino acid RNA-synthetases, codons and ribosomes. These changes result in a translation disturbance, with secondary consequences in cell differentiation, regulation and autonomy. Champy-Hatem [1] admits that a complex is formed between purine bases and 3,4-benzpyrene that results in the synthesis of abnormal nucleic acid.

CONCLUSIONS

1. The transformation of a very high number of normal cells into tumoral cells achieved *in vivo* by the chemical cancerigen 3,4-benzpyrene in the golden hamster is proved by the presence of a wide range of numerical variations and chromosomal restructurings.

2. The diversity of genotypes present at different levels of neoplastic development points to the fact that genetic disturbances form an essential part of the cancerogenesis process.

3. The great karyotype diversity observed by us, the maintaining of numerical variations and of unstable chromosomal restructurings are the cytogenetic expression of the fact that the cancerization process under the influence of 3,4-benzpyrene affects the control mechanisms of cell division. Thus, tumour cells no longer answer to control mechanisms operating in their origin tissue. The organism, trying to protect itself or to direct tumour growth in agreement to it, works out different systems that disturb the evolution of a single tumour clone and result in the appearance of competition.

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REFERENCES

1. Champy-Hatem S., 1969, Bull. Acad. nat. Méd., **153**, *9—10*, 140—148.
2. Di Paolo J.A., Nelson R.S., Donovan P.J., 1971, J. nat. Cancer Inst., **46**, 171—181.
3. Gye W.E., Foulds L., 1939, Amer. J. Cancer., **35**, 108—114.
4. Hauschka T.S., 1961, Cancer Res., **21**, *8*, 957—974.
5. Lesko S.A., 1969, Biochemistry, **8**, *6*, 2291—2298.
6. Lesko S.A., Hoffman H.D., Tso P.O.P., Maher V.M., 1969, Progress in Molecular and Submolecular Biology, **2**.
7. Levan A., 1967, Hereditas, **57**, 343—355.
8. Lungeanu A., Stana A., 1972, Rev. roum. Biol. — Zool. **17**, *6*, 427—429.
9. Ohno S., 1971, Physiol. Rev., **51**, *3*, 496—526.
10. Rei Kato, Levan A., 1969, Jap. J. Genetics, **44**, suppl. 2,90—91.
11. Sandberg A.A., Cancer Res., 1966, **26**, 2064—6080.
12. Weinstein I.B., Cancer Res., 1971, **31**, 651—655.

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